THE BOTANICAL GAZETTE

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THE BOTANICAL GAZETTE

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JOHN MERLE COULTER

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ERRATA

VOL. LXXI

P. 422, line 14, for microchemical read macrochemical

VOL. LXXII

P. 305, footnote, for no. 70 read no. 71

P. 314, line 7 from bottom, also in headings of tables I and III, for ruthenian read ruthenium

P. 318, table II under radish test for callose in sand, for Layer at tip read Same as in loam

VOLUME LXXII NUMBER 1

THE

BOTANICAL GAZETTE

JULY 1921

RESPIRATION OF DORMANT SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 282

HOPE SHERMAN

(WITH FOUR FIGURES)

Introduction

Plant physiologists have long been interested in the physiological processes associated with the development and particularly with the germination of seeds. Much attention has been devoted to those seeds which, when ripe, fail to respond to germinating conditions unless subjected to special treatment or permitted to undergo a distinct rest period. Such dormant seeds offer many problems to pique the curiosity of the investigator, and work on individual seeds has given some conception of the environmental factors influencing dormancy (**4, 15, 17, 40**), as well as of the internal conditions retarding germination and of the chemical changes which take place in after-ripening (**19, 25, 27, 38**). Furthermore, dormant seeds often retain their viability for long periods of time. BEALE (**7, 8, 24, 41**) reported that *Amaranthus retroflexus* will remain viable in the ground for thirty years. If such seeds are fully imbibed, their remarkably prolonged viability may be due either to especially large food reserves or to a tremendous reduction of the rate at which such reserves are respired. A study of the respiration of stored seeds at different time intervals might help to interpret this point.

While the respiration of most plant parts has been studied more or less, there are comparatively few data on resting seeds. For this reason the study of the respiration of seeds would be of interest, per se, and if dormant seeds were selected, it might be possible both to discover differences in the respiration of related and of unrelated species, and, through the acquisition of information upon the rapidity with which food reserves were utilized, to arrive at some idea of the probable longevity of the seeds.

Most dormant seeds belong to one or another of certain main classes (CROCKER 14): those in which the dormancy is due to coat characters, as impermeability to water or to oxygen, acting in conjunction with some physiological character in the embryo aside from actual dormancy; or those in which dormancy is conditioned by the embryo itself, either through lack of differentiation or through the absence of some factor essential for germination, even when the naked embryo is exposed to all ordinary external germination conditions. In addition there are certain seeds with mature embryos whose coats apparently exclude neither water nor oxygen, but still germination is hindered; such delay in *Alisma Plantago* (15) is due to the inability of the embryo to overcome the mechanical resistance to expansion offered by the coats. Finally, dormancy may result from the joint action of two or more of these factors.

In the investigation, some of the results of which are embodied in this paper, the original intention was to study the respiration of each type of dormant seeds, but during the progress of the work the comparative respiration of dormant seeds has become of prime interest, and this phase forms the subject of the present report.

The seeds selected were three on which physiological studies had already been made, but for which respiratory data were lacking, namely, *Amaranthus retroflexus*, *Chenopodium album*, and *Crataegus*,[1] and in addition (because of their economic importance and the ease with which they could be obtained, as well as because of their relationship to *Crataegus*) seeds of the common drupaceous Rosaceae. In *Crataegus* and *Amaranthus* some attempt has been made to determine variations in respiration accompanying after-

[1] The species chiefly studied was *C. coccinea*.

ripening or aging. Furthermore, since many workers claim that catalase activity varies with the respiration, catalase determinations have been made on most of the seeds used.

Methods

Catalase activity was measured by the volume of oxygen set free from hydrogen peroxide (the Oakland Chemical Company's dioxogen) by a given weight of seeds. The apparatus was essentially that described by APPLEMAN (1), and the routine procedure was to grind the seeds for one minute in a mortar with sand and calcium carbonate, add 5 cc. of distilled water, and grind a second minute. The contents of the mortar were then transferred to a bottle which was placed in a water bath at 25° C. The level of the gas burette was adjusted, and after sufficient time had elapsed for the seed suspension to come to the temperature of the bath, the exit of the gas burette was closed. Constancy of level of the water meniscus in the gas burette was assumed to indicate stability of temperature. Thereupon the cock of the dropping funnel was opened on the minute, and 5 cc. of dioxogen was allowed to flow into the bottle, which was immediately set shaking. Readings of the volume of oxygen liberated were taken on the gas burette every minute for the first five minutes and on the tenth minute also.

The respiration determinations were made by means of a respirometer designed by CROCKER (26). This consists of a cylindrical glass chamber fitted with a glass stopper through which pass two tubes, one a manometer and the other a short, straight tube provided with a stopcock by which the chamber can be closed to the surrounding air. Seeds which had been soaked for twenty-four hours and thereafter stored on moist filter paper at about 10° C. were placed in a porcelain hooded holder, designed by HARRINGTON (25). This holder was supported within the respirometer on projections from its wall about 1 cm. above the base. The respirometer was placed in a water bath at constant temperature, and after half an hour the mercury in the manometer was brought to a level and the stopcock closed. When the experiment was to be brought to an end, the difference between the mercury levels in the two arms of the manometer was measured, and a known volume of caustic potash was introduced into the

respirometer through the stopcock tube. The potassium hydroxide was allowed to flow down the sides of the respirometer, the hood of the seed holder preventing its coming into contact with the seeds. So far as possible, measurements were made and absorption carried out without removing the respirometer from the bath. A second reading of the manometer was taken after absorption of the carbon dioxide. Barometer readings were taken after closing the chamber at the beginning, and again before absorption of the carbon dioxide at the end of the experiment. From the calibration of the respirometer it was possible to calculate its volume at the beginning (corrected for the volume occupied by the seeds) and at the end, before and after carbon dioxide absorption, a correction for the volume of the absorbent added being applied in the latter case. All volumes were further corrected to absolute zero, and to 760 mm. pressure. The difference between the volumes at the end of the experiment before and after carbon dioxide absorption represents the volume of carbon dioxide eliminated by the seeds, while the difference between the volume at the beginning of the experiment and that after absorption represents the volume of oxygen taken up by the seeds. These relations are expressed by the following formulae, which were used for the calculation:

Let $V_r \equiv$ volume of respirometer,
$V_s \equiv$ volume of imbibed seeds,
$V_a \equiv$ volume of absorbent (KOH) used,
$T_1 \equiv$ initial absolute temperature,
$T_2 \equiv$ final absolute temperature
$B_1 \equiv$ initial barometric pressure,
$B_2 \equiv$ final barometric pressure,
$m_1 \equiv$ initial manometer reading,
$m_2 \equiv$ final manometer reading.

A: $$(V_r - V_s)\frac{273}{T_1} \times \frac{B_1}{760}$$

B: $$(V_r - V_s)\frac{273}{T_2} \times \frac{B_1 \pm m_1}{760}$$

C: $$(V_r - V_s - V_a)\frac{273}{T_2} \times \frac{B_2 \pm m_2}{760}$$

Then

$$B-C = \text{volume of } CO_2 \text{ eliminated,}$$
$$A-C = \text{volume of } O_2 \text{ absorbed.}$$

From these volumes the respiratory quotient (CO_2/O_2) is easily calculated, as are also the milligrams of carbon dioxide eliminated and of oxygen absorbed per gram of imbibed weight per day, using 1.96 mg. as the weight of 1 cc. of CO_2, and 1.428 mg. as that of 1 cc. of O_2.[2]

[2] The application of these formulae is illustrated in the following calculation of data from an experiment on *Amaranthus:*

$V_f = 24.61$ cc.	$T_1 = 25°$ C.	$B_1 = 751.5$ mm.	$m_1 = -\ 9$ mm.
$V_s = 1.00$ cc.	$T_2 = 25°$ C.	$B_2 = 749.8$ mm.	$m_2 = -65$ mm.
$V_a = 0.76$ cc.			

Weight imbibed seeds = 1.3177 gm.; duration of experiment = 24 hours.

$$\begin{array}{r} 24.61 \\ -\ 1.00 \\ \hline 23.61 \end{array} \times \frac{751.5}{760} \times \frac{273}{298} = 21.387 \qquad \text{(A)}$$

$$23.61 \times \frac{740.8}{760} \times \frac{273}{298} = 21.082 \qquad \text{(B)}$$

$$\begin{array}{r} -\ 0.76 \\ \hline 22.85 \end{array} \times \frac{684.8}{760} \times \frac{273}{298} = 18.862 \qquad \text{(C)}$$

$$\text{Log. } \frac{273}{760 \times 298} = 7.081133 - 10$$

log. 23.61 = 1.373096	log. 23.61 = 1.373096	log. 22.85 = 1.358886
log. 751.5 = 2.875929	log. 740.8 = 2.869701	log. 684.8 = 2.835564
-3.081133	-3.081133	-3.081133
1.330158	1.323930	1.275583
Antilog. 1.330158 = 21.387	Antilog. 1.323930 = 21.082	Antilog. 1.275583 = 18.862

$B-C = 21.082 - 18.862 = 2.220$ cc. CO_2; $A-C = 21.387 - 18.862 = 2.525$ cc. O_2;
$CO_2/O_2 = 0.880$.

log. 2.220 = 0.346353	log. 2.525 = 0.402261
log. 1.96 = 0.292256	log. 1.428 = 0.154728
colog. 1.3177 = 0.880183	colog. 1.3177 = 0.880183
0.518792	0.437172

antilog. 0.518792 = 3.302 mg. CO_2 per gm. imbibed weight per 24 hours.
antilog. 0.437172 = 2.736 mg. O_2 per gm. imbibed weight per 24 hours.

Investigation

The material studied was as follows:

Seeds	Year of crop	Time of collection
Amaranthus retroflexus	1919	August 2–September 7, 1919
Chenopodium album	1918	January 29, 1919
Rumex crispus	1919	August 1919
Crataegus	1917	October 1917

In addition, seeds of *Prunus pumila* (from Mineral Springs, Indiana), and of *P. persica*, *P. armeniaca*, *P. Cerasus* var. *Morella*, *P. domestica* var. Blue Gage, and the red Burbank plum, obtained in the market, were also studied. All rosaceous seeds except *Crataegus* were freed at once from pulp, dried, and in most cases opened and used immediately. *Amaranthus* and *Chenopodium* seeds were stored at room temperature until used. Seeds of *Crataegus* were left at room temperature until they were removed from the carpels, when they were placed at once under germinating conditions at 10° C.

In preparation for an experiment the seeds were placed in distilled water and left in the refrigerator at approximately 10° C. for twenty-four hours. Cotton and filter paper were placed in Petri dishes and the whole sterilized in an electric oven. Before being used, the cotton was saturated with sterile distilled water. The seeds were thoroughly shaken in several portions of sterile water, and were either used at once or placed in the Petri dishes and stored in the refrigerator until needed, in order to avoid the influence on respiration of variations of temperature. By means of these precautions it was possible, to a very great extent, to prevent infection of the seeds with molds or bacteria, and at the same time to avoid the modification of respiration due to treatment with disinfectants (**36, 38, 42**). The amount of material used depended largely upon the size of the seed and of the apparatus. For *Amaranthus* and *Chenopodium*, 1 gm. of air-dry seeds was the usual amount, a weight representing approximately 1000 seeds. Corresponding numbers and weights for the other seeds were:

Seed	Number	Weight (gm.)
Rumex crispus	.....	0.5
Prunus persica	2	0.7 –1.00
Prunus domestica	2	0.7 –0.8
Prunus armeniaca	1	0.8+
Prunus Cerasus	10	0.8
Prunus pumila	10	0.8
Crataegus	50–200	0.7–3.00

With the use of large numbers of seeds, possible in the case of the small seeds of *Amaranthus*, *Chenopodium*, and *Rumex*, individual variations are abolished, and the results are probably more nearly typical than those obtained by the use of one or two seeds, where individual peculiarities would assume an exaggerated significance. From four to ten lots of seeds were run at the same time, since the variability in oxygen consumption and in carbon dioxide elimination was early evident, and it was only by running at least four experiments simultaneously, under precisely similar conditions, that variability could be limited to factors intrinsic in the seed.

The experimental temperature (with rare exceptions) was either 20° C. or 25° C., and the results are all corrected to a comparable basis. The average duration of the experiments was 24 hours. Occasionally a longer time interval was employed, but rarely a shorter, as the amounts of gas absorbed and eliminated were usually small. The average amount of carbon dioxide eliminated during an experiment was 2 cc., with a maximum of 4 cc. The volume of the respirometers was approximately 25 cc. Since KIDD (28) found that 10 per cent of carbon dioxide retards respiration, the accumulation of this gas during an experiment may have slightly modified at times the character of the respiration.

CATALASE DETERMINATION

Table I is a comparison of the catalase activity in the different seeds studied. The variability in catalase activity is extreme,

TABLE I

CATALASE ACTIVITY OF SEED IMMEDIATELY AFTER HARVESTING

SEEDS	WEIGHT OF MATERIAL (GM.)	CONDITION	OXYGEN (CC.) LIBERATED AFTER			
			1 minute	3 minutes	5 minutes	10 minutes
Amaranthus retroflexus.	0.13	Imbibed	3.20	6.10	7.67	8.97
Chenopodium album...	0.13	Imbibed	3.00	6.05	7.40	7.30
Apricot.....	0.13	Imbibed	10.10	35.10	48.20	56.50
Blue gage plum.....	0.13	Imbibed	7.90	21.40	28.80	35.60
Blue gage plum.....	0.13	As removed from carpel	2.65	9.93	12.68	16.55
Burbank plum.....	0.13	As removed from carpel	2.12	7.85	9.72	11.70
Crataegus.....	0.0449	As removed from carpel	4.50	10.50	14.30	18.30
Crataegus.....	0.0688	Imbibed	7.50	18.00	23.10	27.60

especially among the rosaceous seeds. Of these, the greatest activity occurred in apricot, 48 cc. of oxygen being liberated from hydrogen peroxide in five minutes by 0.13 gm. of imbibed seeds. The red Burbank plum had the lowest activity of any rosaceous seed, an equal weight of imbibed seeds liberating only 9.7 cc. of oxygen. In table II the catalase activity of hawthorn is given by periods from the fourth to the forty-second day, while the same

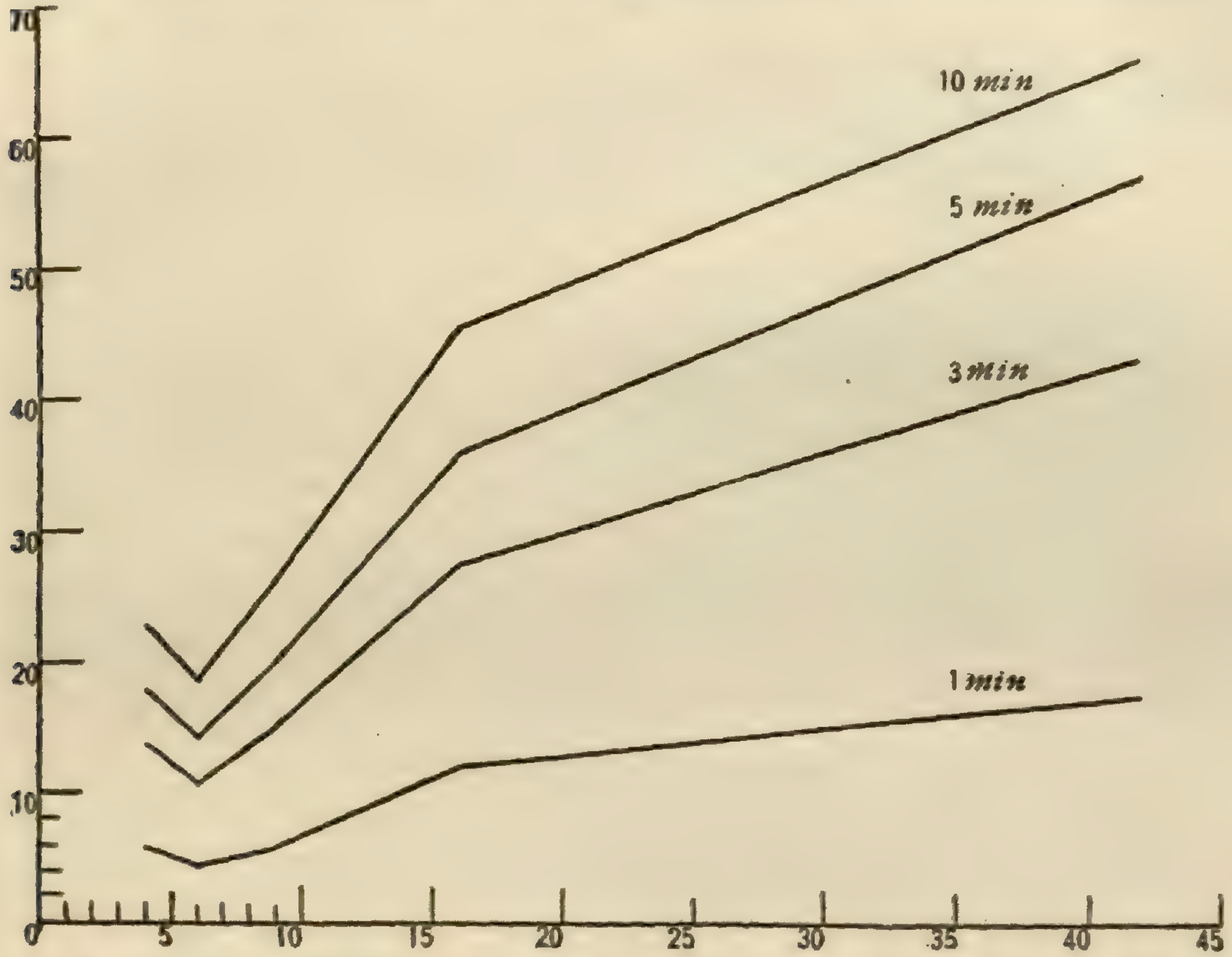

FIG. 1.—Curves of catalase activity at 1, 3, 5, and 10 minute intervals in *Crataegus:* horizontal axes represent time in days after harvesting; vertical axes represent cc. of O_2 liberated; temperature 25° C.

data are presented graphically in fig. 1. There is an increase in catalase activity as after-ripening progresses. A determination made on the after-ripened seeds, 128 days at 10° C., suggests that this increase continues after the forty-second day, but at a very slow rate. This slowness of increase was observed by ECKERSON (19) in her microchemical study of after-ripening in *Crataegus.* The stability of catalase activity in *Amaranthus* during the first month after harvesting is plain from table III.

TABLE II

CATALASE ACTIVITY IN *Crataegus* DURING DORMANCY

NUMBER OF DAYS AT 10° C.	WEIGHT OF MATERIAL (GM.)	OXYGEN (CC.) LIBERATED AFTER			
		1 minute	3 minutes	5 minutes	10 minutes
4.....	0.0410	5.8	13.7	17.9	22.9
6.....	0.0690	4.4	10.6	14.3	18.8
9.....	0.0778	5.7	15.0	20.0	26.2
16.....	0.0773	11.9	27.2	36.0	45.5
42.....	0.0791	17.3	42.9	57.0	65.8
128.....	0.1007	25.6	57.2	65.2*	67.0

*There are two possible explanations for the small increase in catalase activity from the 42nd to the 128th day: (1) the amount of oxygen liberated may have been limited by the use of only 5 cc. of dioxogen; (2) a determination should have been made at 90 days, when after-ripening was complete. After that time the seed may go into a secondary dormancy (**18**).

TABLE III

CATALASE ACTIVITY IN *Amaranthus retroflexus* (0.13 GM. IMBIBED SEEDS USED)

NUMBER OF DAYS AFTER COLLECTION	OXYGEN (CC.) LIBERATED AFTER			
	1 minute	3 minutes	5 minutes	10 minutes
8.....	3.0	6.2	7.9	9.2
16.....	2.5	5.0	6.2	7.3
28.....	3.4	6.2	7.7	9.0
44.....	3.8	7.2	8.9	10.4

RESPIRATION

Table IV embodies the comparative respiratory behavior of all ten seeds. While all but *Rumex* have a respiratory quotient less than unity, indicating an oxygen intake in excess of the carbon dioxide elimination, the value of the carbon dioxide-oxygen ratio varies within wide limits. For the Rosaceae the "respiratory intensity," as measured by the milligrams of carbon dioxide eliminated per hour per gram of imbibed seeds, averages about 0.08, while in the other seeds it is higher, being about 0.11 in *Amaranthus*, and 0.15+ in *Rumex* and *Chenopodium*. This difference may be due to the character of the storage substance, chiefly starch (**44**), present in the last three seeds, but it is undoubtedly also attributable in part to a difference in degree of dormancy.

Only one set of experiments was carried out on *Rumex*, because it was found that even immediately after harvesting the seeds would germinate. In many of the seeds the coats were ruptured and the hypocotyls emerging by the end of the experiment. *Chenopodium* also exhibited a marked readiness to germinate. *Amaranthus* was more dormant, but even within a few weeks of harvesting, on removing the seeds from the respirometer after an experiment, an occasional seed with coat broken was found; while during the later experiments, over 100 days after harvesting, the number with broken coats increased greatly (80 seeds per 1000). The

TABLE IV

RESPIRATORY VALUES (AT 25° C.)

Seeds	No. of experiments	CO_2/O_2	Mg. CO_2 per 24 hours per gm. imbibed weight	Mg. O_2 per 24 hours per gm. imbibed weight
Amaranthus retroflexus....	37	0.856	2.691	2.324
Chenopodium album.....	14	0.928	4.213	3.307
Rumex crispus.....	9	1.160	3.636	2.291
Crataegus.....	56	0.774	1.548	1.474
Peach.....	14	0.675	1.881	2.033
Apricot.....	30	0.648	2.106	2.392
Cherry.....	19	0.866	2.589	2.186
Sand cherry.....	19	0.876	2.288	1.935
Blue gage plum.....	19	0.696	2.579	2.748
Burbank plum.....	10	0.912	1.998	1.610

rosaceous seeds are really dormant. *Crataegus* requires three months of after-ripening at low temperature (5° C. optimum) before the hypocotyl emerges from the coat. The changes occurring during after-ripening in *Crataegus* progress slowly (**19**) until very near the end of dormancy. The data reported represent determinations covering the period from the first to the seventy-seventh day under germinating conditions. At this latter time the seeds are still dormant and would fail to germinate if removed to a higher temperature. For the other rosaceous seeds no attempt was made to determine the exact duration of dormancy, although it was observed that seeds left in the refrigerator germinated in from 1.5 to 3 months.

It has been suggested (**17**) that the dormancy of *Crataegus*, although chiefly conditioned by the embryo, is in part dependent

upon the coats, which reduce the rate of imbibition and perhaps of oxygen entrance. The effect of an atmosphere entirely oxygen was accordingly determined, and it was found that the quotient and the respiratory intensity of the dormant seed still fluctuated. The mean respiratory quotient was a trifle lower than in ordinary air, 0.728 instead of 0.774. Further investigation of this point will determine the effect of varying percentages of oxygen upon the dormant seed and on the after-ripened as well. Although a detailed study of the respiration of the after-ripened seed is yet to be made, data already obtained seem to indicate that the respiratory quotient and the milligrams of carbon dioxide eliminated are

TABLE V

RESPIRATION OF *Amaranthus retroflexus* (AT 25° C.)*

Number of days after harvesting	CO_2/O_2	Mg. CO_2 per 24 hours per gm. imbibed weight	Mg. O_2 per 24 hours per gm. imbibed weight
3	0.824	2.425	2.150
11	0.850	2.263	1.938
24	0.892	2.299	1.877
35	0.890	2.932	2.417
40	0.854	1.955	1.656
71	0.877	2.705	2.591
104	0.802	4.475	4.056
140	0.885	3.979	3.500
176	0.842	1.794	1.537

* Seeds stored at room temperature until used.

slightly higher than in the dormant seed, while the oxygen absorption is lower. The effect of increased percentages of oxygen on after-ripened apple seed is to increase its respiratory intensity (**25**). It may be that this will be found to be the effect on the after-ripened hawthorn.

The values given for *Amaranthus* in table IV are averages based on experiments on seeds at intervals from 3 to 176 days after harvesting. In table V these respiratory values are given by periods, while in fig. 2 the carbon dioxide-oxygen ratio, and the respiratory intensity, as indicated by milligrams of carbon dioxide eliminated as well as of oxygen absorbed, are plotted in a time curve. The uniformity of the carbon dioxide-oxygen ratio is noticeable. The high values for the 104th and 140th days are accompanied by

a slight increase in germination. Still more interesting facts are brought out by the frequency histograms (fig. 3), from which are evident the value most frequently appearing for the carbon dioxide-oxygen ratio, and the total variation of this ratio in the entire number of experiments on each seed. In plotting these histograms the values for the quotients were grouped, and since it was found that experimentally and mathematically the digits for the quotient

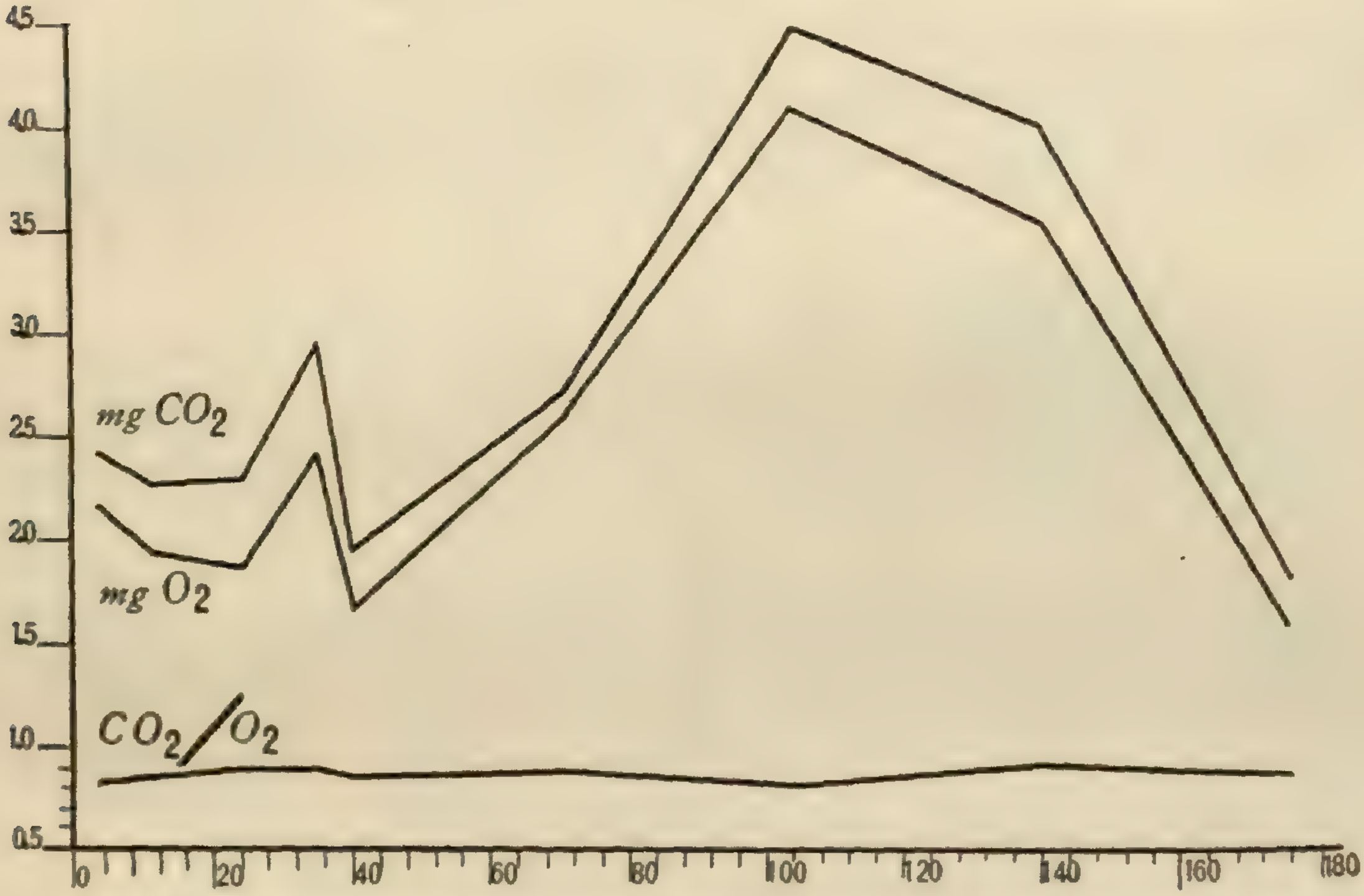

FIG. 2.—Respiration of *Amaranthus retroflexus* under storage at room temperature: horizontal axes represent time in days after harvesting; vertical axes represent values from 0.5 to 4.5 for the CO_2/O_2, these being absolute numbers indicating ratio; for "respiratory intensity" curves represent mg. of gas per gram imbibed weight of seeds per 24 hours.

are significant only to hundredths, the interval between these groups or classes was taken as 0.01.

The range of the value of this ratio, as determined by the maximum and minimum, varies widely in the different seeds, being least in *Amaranthus* (0.685–0.975, that is, 29 classes) and widest in hawthorn (0.470–1.140, that is, 67 classes). In *Chenopodium* the heaviest grouping lies within a range of only seven classes, but between the lower limit of this group and the next lowest quotient is a gap of nineteen classes, while above the group's highest limit

FIG. 3.—Frequency distribution of respiratory quotient in *Crataegus*, *Amaranthus*, and *Chenopodium*: horizontal axes represent class in which respiratory quotients fall; vertical axes represent number of experiments whose quotients fall in given class. Class interval for *Crataegus* 0.01 (0.47–0.48); mean 0.7905; mean deviation 0.079, hence majority of such experiments will tend to vary from 0.79 by 0.08; standard deviation 0.172. Class interval for *Amaranthus* 0.01 (0.685–0.695); mean 0.8578; mean deviation 0.04; standard deviation 0.05. Class interval for *Chenopodium* 0.01 (0.685–0.695); mean deviation 0.02; standard deviation 0.04; mean 0.9431; mean deviation 0.02; standard deviation 0.04.

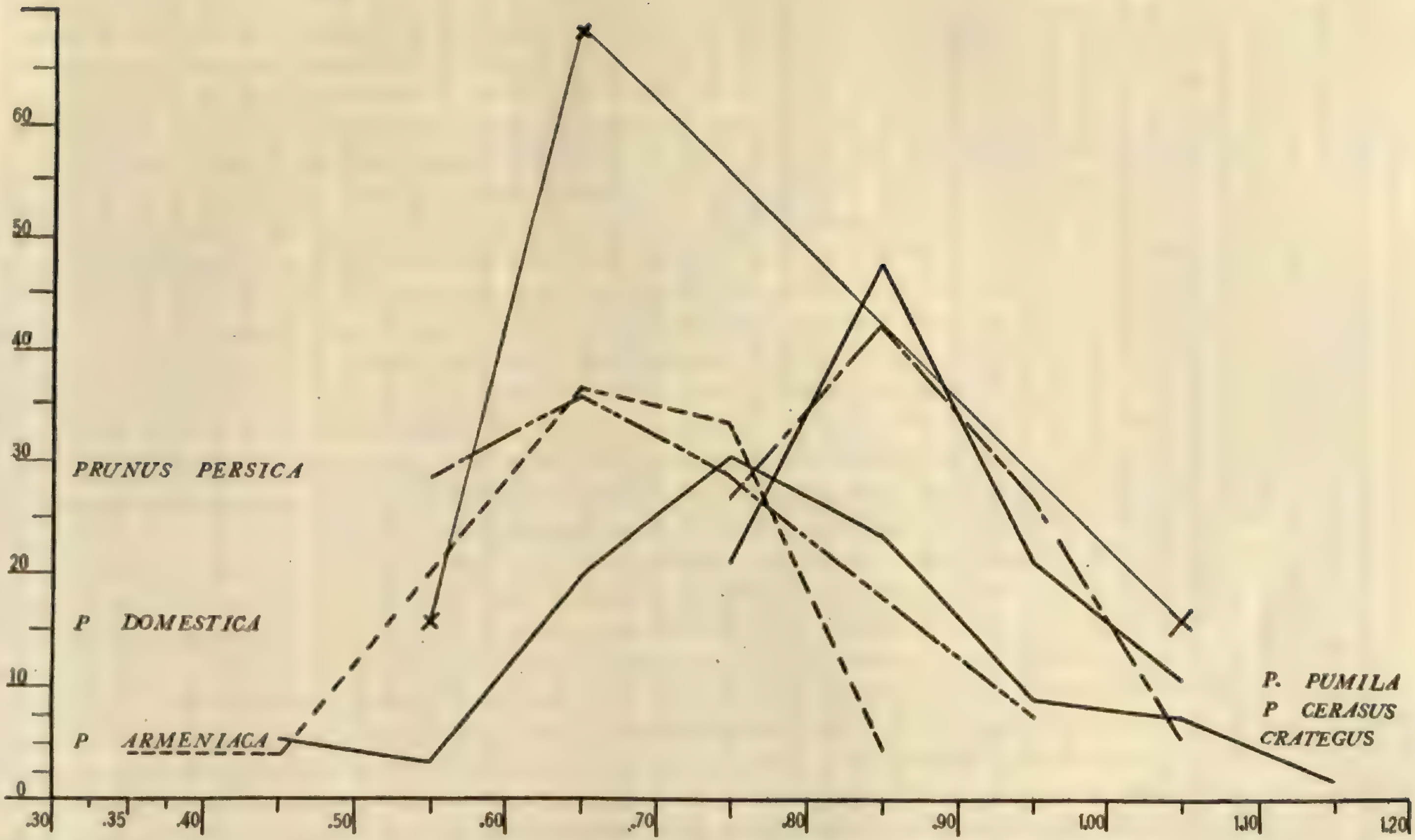

FIG. 4.—Comparison of respiratory quotients in Rosaceae; vertical axes indicate percentages of experiments in which each quotient value occurs; horizontal axes indicate quotient values; grouping is by tenths (from 0.1–0.2, etc.); maximum for peach, apricot, and blue gage plum lies between 0.6 and 0.7 CO_2/O_2 value; for hawthorn between 0.7 and 0.8; for cherry and sand cherry between 0.8 and 0.9.

are five quotients grouped discontinuously. The irregularity and discontinuity of grouping lay the extreme values open to question from the mathematician's point of view. In this instance experimental evidence supports the mathematician's feeling that certain unusual factors must be working to produce the higher values, inasmuch as these were obtained in experiments on seeds of the 1919 crop, tested within two days after their collection. The seeds were not entirely freed from chaff and adherent scales, and in each set mold developed freely during the experiment. The high values obtained, therefore, represent the joint respiratory activity of seeds and mold. These values have been allowed to stand in the histogram to illustrate the value of such treatment of data as a test of its uniformity, but they are not used in calculating the average for the type quotient in table IV.

In fig. 4 the values for the respiratory quotients of the Rosaceae are plotted in a percentage curve, the abscissas representing the value of the quotients, and the ordinates representing the percentage of the total number of experiments on each seed, in which each value occurred. The maximum percentage of the experiments with the six rosaceous seeds, apricot, peach, cherry, sand cherry, blue gage plum, and hawthorn, gives respiratory quotients lying between 0.60 and 0.90, the extreme range of the means for the different seeds (table IV) being twenty-three classes, 0.648–0.876. Within this range the maxima fall into three groups: those of cherry and sand cherry, between 0.80 and 0.90; those of peach, apricot, and blue gage plum, between 0.60 and 0.70; while that of hawthorn lies intermediate between these values (0.70–0.80). Thus the maximum for hawthorn falls very close to 0.756, the mean of the quotients (table IV) for these six seeds; and with the exception of a single value for apricot all the quotients for these rosaceous seeds lie within the range of the quotients of hawthorn.

Discussion

Increase in catalase activity during after-ripening of seeds and during germination has been reported by numerous workers. ECKERSON (19), by microchemical methods, found an increase in the activity of catalase during the after-ripening of *Crataegus*, and

in the present investigation the same phenomenon was observed macroscopically, under after-ripening and germinating conditions. Such an increase during after-ripening is characteristic of seeds with dormant embryos.

On the other hand, in *Amaranthus retroflexus* catalase activity appears to be far less subject to fluctuation. CROCKER and HARRINGTON (16) find surprisingly slight variation in the catalase activity during after-ripening, which in *Amaranthus* occurs "during the first three or four months in dry storage." The activity for the first month and a half after harvesting, as shown in table III, is maintained at a very uniform rate. A comparison of the values obtained on the imbibed seeds with those found by CROCKER and HARRINGTON for the dry powder indicates the uniformity of the degree of activity in different seeds:

WEIGHT OF POWDER (GM.)	OXYGEN (CC.) LIBERATED AFTER		
	1 minute	5 minutes	10 minutes
0.10 dry powder.....	4.9	9.0	11.1
0.13 imbibed seeds...	3.9	6.1	7.1

The values for the dry seeds, however, are slightly higher than for the imbibed, probably owing to the greater concentration of material in a given weight. In this connection the results obtained by CROCKER and HARRINGTON on samples of *Amaranthus* collected in 1894 are of interest. They found the catalase activity of these twenty-three year old seeds but little diminished, although there was complete loss of viability.

DATE OF COLLECTION	OXYGEN (CC.) LIBERATED AFTER			PER CENT GERMINATION AFTER 7 DAYS
	1 minute	5 minutes	10 minutes	
1917 (average of 3 samples).	8.3	20.0	23.8	100
1894 (average of 3 samples).	7.8	17.8	20.8	0

The extreme stability of the catalase activity is emphasized by the fact that one 1894 sample gave values identical with those obtained from one of the 1917 samples:

Date of collection	Location	Oxygen cc. liberated after			
		1 minute	3 minutes	5 minutes	10 minutes
1917.....	Pullman, Wash.	8.7	18.1	22.7	26.8
1894.....	East Lansing, Mich.	8.7	18.1	22.7	26.8

In the seeds studied in the present investigation, the greatest degree of activity was manifested by the rosaceous seeds (seeds having dormant embryos).

Many plant and animal physiologists have been inclined to postulate a parallelism between catalase activity and respiratory intensity (**2, 3, 10, 11, 12, 13**). In *Acer saccharum* (**27**) and *Juniperus virginiana* (**38**) both catalase activity and respiratory intensity increase as dormancy ends and germination begins. In *Crataegus* catalase activity increased continuously up to the twelfth day in the germinator (the time of the last determination), but the increase was not uniform. Respiratory intensity increased up to the sixth day. From that time to the seventy-seventh day it tended to decrease, but at an irregular rate and with considerable fluctuation. In *Amaranthus* the respiration, like the catalase activity, is maintained at a relatively uniform rate for some time (176 days), but fluctuations in the one are not coincident with fluctuations in the other, and at times may be in an opposite direction. These facts are in harmony with the decision to which their own studies led Crocker and Harrington, that "in *Amaranthus* seeds there is no evidence of a correlation between catalase activity and respiratory intensity."

That high catalase activity does not necessarily accompany a high respiratory quotient or respiratory intensity (as indicated by milligrams of carbon dioxide eliminated) is evident when the seeds studied are arranged in descending order of these values, as given in table VI.

Although there are relatively few determinations of respiratory values for resting seeds, the literature is rich in findings for other plant parts. A comparison of these values, however, is often difficult because of their variable form and the frequent absence of data necessary for the determination of measured and calculated values.

In table VII results selected from numerous investigations have been recast in such form as to make them comparable with the results on resting seeds obtained in the present study.

In the following discussion of the respiration studies the results are treated as they stand. It is recognized that the temperature at which the experiments were carried out (20°–25° C.) was high, and undoubtedly led to more vigorous respiration than occurs at 10° C. The transfer from the latter temperature, at which the seeds were stored when under after-ripening or germinating conditions, to the higher temperature of the water bath, may in

TABLE VI

Catalase activity	Respiratory intensity		CO_2/O_2
	Mg. CO_2 eliminated	Mg. O_2 absorbed	
1. Apricot	Chenopodium	Chenopodium	Rumex
2. Blue gage plum (imbibed)	Rumex	Blue gage plum	Chenopodium
3. Crataegus (imbibed)	Amaranthus	Apricot	Burbank plum
4. Crataegus (as removed from carpels)	Cherry	Rumex	Sand cherry
5. Amaranthus	Blue gage plum	Cherry	Amaranthus
6. Chenopodium	Sand cherry	Amaranthus	Cherry
7. Blue gage plum (as removed from carpels)	Apricot	Peach	Crataegus
8. Burbank plum	Burbank plum	Sand cherry	Blue gage plum
9.	Peach	Burbank plum	Peach
10.	Crataegus	Crataegus	Apricot

itself have accelerated respiration. In the case of apple, HARRINGTON (**25**) finds great diminution of the respiratory intensity at low temperature. An investigation of the respiratory intensity of the seeds used in the present instance for ten degree intervals of temperature will throw light on this point.

The problem of the longevity of seeds is still unsolved, although various theories have been advanced. Loss of vitality might result from exhaustion of stored food, degeneration of enzymes, accumulation in respiration or digestion of substances toxic to the seed, or from still other internal changes in the seed substance inimical to its life. GROVES (**14, 23**) found that life duration of *Triticum sativum* was a logarithmic function of the temperature, and LEPESCHKIN'S time-temperature formula for the coagulation of

TABLE VII

Comparative respiration of different plant organs

Organ	Reference	Temperature (°C.)	CO_2/O_2	Mg. CO_2	Mg. O_2
				Per hour per gm. fresh (or imbibed) weight	
Seedlings					
Triticum	5	13	0.98	55.92	41.54
Ricinus communis	5	20	0.96	37.67	33.54
Lupinus	22	8	1.03	36.49	25.56
Stems of succulents					
Sedum reflexum					
Large stems (day)	5	31	0.98	23.83	17.70
Large stems (night)	5	23	0.88	17.05	14.14
Opuntia tomentosa					
Very young (day)	5	25	0.77	3.86	3.83
Very young (night)	5	23	0.047	0.412	6.40
Stamens					
Antirrhinum majus					
Anthers	32	24	0.87	2.86	5.62
Filaments	32	24	1.02	1.32	2.32
Entire	32	20	0.93	0.606	0.457
Acanthus mollis					
Adolescent	32	21	0.91	1.027	0.806
Young	32	21	0.97	0.640	0.482
Adult	32	21	0.79	0.538	0.518
Entire	32	20	0.81	0.546	0.589
Seeds					
Acer saccharum	27	.....	.....	0.27	.....
Petals					
Antirrhinum majus					
Young	32	24	1.15	1.613	1.455
Adolescent	32	24	1.13	1.374	0.970
Adult	32	24	1.00	0.668	0.487
Acanthus mollis					
Young	32	26	0.79	1.088	0.952
Adolescent	32	26	0.83	0.717	0.606
Adult	32	26	0.94	0.633	0.485
Leaves of seedlings					
"Bean" (Phaseolus?)	22	.....	.....	1.54	.....
Zea Mays					
Green leaves	37	26	0.99	1.339	0.979
Etiolated leaves	37	26	0.97	1.060	0.795
Vicia Faba					
Green leaves	37	21	0.90	1.226	0.988
Etiolated leaves	37	21	0.87	0.956	0.794
Leaves of Ligustrum japonicum					
Variegated leaves					
Green parts*	37	26	0.84	1.331	1.145
White parts	37	26	0.80	1.047	0.958
Germinating seeds					
Phaseolus vulgaris	30	.....	.....	1.276	.....
Embryo					
Hordeum vulgare	42	24	1.09	1.237	.....

* In a microchemical examination of numerous variegated leaves, Eckerson found oxidases, peroxidases, and catalases higher in the green than in the white portions.

TABLE VII—*Continued*

Organ	Reference	Temperature (°C.)	CO_2/O_2	Mg. CO_2	Mg. O_2
				Per hour per gm. fresh (or imbibed) weight	
Pistils					
Antirrhinum majus, entire	32	20	1.00	1.186	0.864
Acanthus mollis					
Young	32	21	0.89	0.609	0.492
Adolescent	32	21	0.87	0.531	0.428
Entire	32	20	0.84	0.490	0.409
Adult	32	21	0.90	0.486	0.387
Germinating seeds					
Lupinus albus	30	.....	.....	0.990	.....
Vicia Faba	30	.....	.....	0.970	.....
Pisum sativum	30	.....	.....	0.946	.....
Cucurbita Pepo	30	.....	.....	0.878	.....
Flowers					
Syringa vulgaris	22	20	.....	0.788	.....
Leaves and leaflike structures					
Sepals of Acanthus mollis					
Adolescent	23	24	1.03	0.798	0.565
Young	33	24	1.06	0.562	0.858
Adult	33	24	0.88	0.554	0.444
Leaves					
Triticum sativum seedling					
Green	37	25	0.97	0.788	0.588
Etiolated	37	25	0.98	0.735	0.431
Hordeum vulgare seedling					
Green	37	23	0.85	0.521	0.444
Etiolated	37	23	0.83	0.423	0.371
Acanthus mollis	37	22	0.77	0.361	0.212
Antirrhinum majus	37	24	0.73	0.354	0.182
Antirrhinum majus	37	22	0.88	0.314	0.251
Malva sylvestris	37	15	0.71	0.251	0.246
Syringa vulgaris	9	22	0.94	.....	.....
Syringa vulgaris	33	22	0.94	.....	.....
Taxus baccata	9	16	0.86	.....	.....
Pinus maritima	9	20	0.85	.....	.....
Pinus sylvestris	9	24	0.80	.....	.....
Eucalyptus	9	19	0.80	.....	.....
Leaf blades					
Vicia sativa	37	18	0.75	0.590	0.574
Rumex pulcher	37	17	0.76	0.288	0.276
Geranium Robertianum	37	18	0.72	0.272	0.275
Bryonia dioica	37	17.5	0.65	0.259	0.290
Leaf petioles					
Vicia sativa	37	18	0.88	0.327	0.207
Bryonia dioica	37	17.5	0.87	0.184	0.154
Geranium Robertianum	37	18	0.91	0.086	0.068
Rumex pulcher	37	17	0.80	0.064	0.058
Tendrils					
Vicia sativa	37	18	0.90	0.504	0.408
Bryonia dioica	37	17.5	1.02	0.221	0.157
Cladodes					
Asparagus albus	37	18	0.78	0.437	0.410
Ruscus hypophyllum	37	15	0.55	0.051	0.067

TABLE VII—*Continued*

Organ	Reference	Temperature (°C.)°	CO_2/O_2	Mg. CO_2	Mg. O_2
				Per hour per gm. fresh (or imbibed) weight	
Phyllodes					
Acacia megaloxylon	37	18	0.66	0.233	0.257
Entire plant					
Pelargonium zonale	22	12–14	0.54	.....	.....
Sedum hybridum	22	25–26	0.37	.....	.....
Buds					
Aesculus					
Influence of warm bath	35	20	.....	0.188	.....
14 hours in water at 38° C.	35	20	.....	0.138	.....
14 hours in water at 20° C.	35	20	.....	0.122	.....
Stems					
Vicia sativa	37	18	0.86	0.292	0.247
Rumex pulcher	37	17	0.85	0.231	0.197
Asparagus albus	37	18	0.96	0.221	0.167
Accacia megaloxylon	37	18	0.82	0.206	0.182
Bryonia dioica	37	17.5	0.91	0.167	0.133
Geranium Robertianum	37	18	0.94	0.127	0.097
Mesembryanthemum nodiflorum	37	15.5	1.00	0.076	0.055
Ruscus hypophyllum	37	15	0.58	0.033	0.414
Bulbs					
Convallaria					
Untreated	35	19	.....	0.174	.....
After 8 hours in water at 38° C.	35	19	.....	0.133	.....
After 8 hours in water at 18° C.	35	19	.....	0.117	.....
Imbibed seeds					
Juniperus					
130 days at 5° C.	38	25	0.95	0.489	0.4398
100 days at 5° C.	38	25	0.68	0.2486	0.3192
90 days at 5° C.	38	25	0.97	0.2354	0.2092
60 days at 5° C.	38	25	0.97	0.2352	0.2152
30 days at 5° C.	38	25	0.94	0.218	0.1976
5 days at 5° C.	38	25	0.84	0.1311	0.1347
Chenopodium album	Table IV	20	0.928	0.175	0.141
Rumex crispus	"	20	1.16	0.151	0.095
Amaranthus retroflexus	"	20	0.857	0.113	0.096
Prunus cerasus var. Morello	"	20	0.814	0.108	0.091
P. domestica (blue gage)	"	20	0.695	0.107	0.109
P. pumila	"	20	0.878	0.093	0.076
P. armeniaca	"	20	0.628	0.089	0.102
P. domestica (Burbank plum)	"	20	0.912	0.083	0.067
P. persica	"	20	0.675	0.078	0.084
Crataegus	"	20	0.774	0.064	0.061
Zea Mays					
Embryo	42	20	0.83	0.444	.....
Endosperm	42	21	0.55	0.014	.....
Aleurone	42	.....	.....	0.009	.....
Pure endosperm	42	22	0.73	0.004	.....
Intact seed	42	18	0.77	0.003	.....
Hordeum vulgare					
Intact seed	42	26	0.83	0.203	.....
Endosperm (summer)	42	23	0.68	0.078	.....
Aleurone (summer)	42	.....	.....	0.052	.....
Endosperm (winter)	42	15	0.58	0.019	.....

TABLE VII—*Concluded*

Organ	Reference	Temperature (°C.)	CO_2/O_2	Mg. CO_2	Mg. O_2
				Per hour per gm. fresh (or imbibed) weight	
Pure endosperm (summer)	42	23	0.36	0.019	.....
Pure endosperm (winter)	42	20	0.39	0.007	.....
Aleurone (winter)	42	.....	.....	0.001	.....
Ricinus	42	18	0.59	0.082	.....
Tubers					
Solanum					
Entire	22	.....	.....	0.006	.....
5 hours after quartering	22	.....	.....	0.480†	.....
Untreated	35	17–19	.....	0.013	.....
After 6 hours in water at 19.5° C.	.....	.....	.....	0.010	.....
After 5 hours in water at 40° C.	35	.....	.....	0.018	.....
After storage at 0° C. (starch changed to sugar)	35	19	.....	0.059	.....
After 8 hours 2 per cent aqueous ether	35	19	.....	0.068	.....
Fruits					
Russet apple	21	18	1.09	0.019	0.014
Orange	21	18	1.06	0.078	0.164
Japanese plum	21	18	1.30	0.274	0.160
Dry seeds					
Triticum vulgare					
19 per cent water	43	.....	.....	0.0054	.....
Soft red winter wheat (13 per cent water)	6	.....	.....	0.00024	.....
Secale cereale					
19 per cent water	43	.....	.....	0.0001	.....
Zea Mays					
19 per cent water	43	.....	.....	0.0001	.....
Juniperus dry seeds	38	25	0.76	0.00098	0.0011
Hordeum distichum					
33 per cent water	29	.....	.....	0.0001	.....
19–20 per cent water	29	.....	.....	0.00015	.....
15 per cent water	29	.....	.....	0.00004	.....
Algae					
Nostoc	9	19	0.40	.....	.....
Fucus	9	14–15	0.50	.....	.....
Fungi					
Aspergillus niger, Raulin's solution					
Vegetative mycelium (morning)	39	.....	1.04+	.....	.....
Vegetative mycelium (evening)	39	.....	1.05	.....	.....
Fruiting mycelium (evening)	39	.....	1.07	.....	.....
Water and 0.4 per cent salts, black conidia	39	23.5	0.66	.....	.....
Sterigmatocystis nigra					
In solutions containing					
2 gm. tannin	21	.....	1.09	.....	.....
0.9 gm. sugar	21	20	0.93	.....	.....
0.984 gm. tartaric acid	21	.....	1.78	.....	.....
0.75 gm. citric anhydride	21	33	1.40	.....	.....
0.88 gm. malic acid	21	20	1.50	.....	.....
0.80 gm. citric acid	21	20	1.48	.....	.....

† Cf. MAGNESS (31): "Removal of the epidermis facilitates the entrance of oxygen to the tissues and escape of carbon dioxide. It would be interesting to know to what extent the increased respiration following wounding is due to mechanically facilitating gaseous exchange and to what extent it is due to actual metabolic changes in the wounded tissues."

proteins was applicable as a temperature-life duration formula for wheat grains, as LEPESCHKIN himself had found it applicable for imbibed cells. Loss of viability in air-dry seeds, therefore, is probably due to "a time-temperature denaturing of certain colloids (probably proteins) of the embryo" (**16**). The retarding effect of carbon dioxide upon germination has been shown by KIDD (**28**). On the other hand, enzymes may persist and have a high degree of activity in seeds which are no longer viable, as in *Amaranthus*, or their activity may be greatly decreased without marked decrease in percentage of germination, as in Johnson and Sudan grasses (CROCKER **16**). Exhaustion of stored food cannot be considered a cause for decreased life duration in air-dry seeds, but in the case of seeds lying in the soil the situation is different. Such seeds would have a high water content, favoring chemical action, whether respiration or digestion. The actual occurrence of such reactions of course would depend upon oxygen supply, temperature, enzymes present, and the extent to which by-products (carbon dioxide, etc.) were removed. In such seeds, of which *Amaranthus* is a typical example, the life duration might easily be limited by the amount of stored substance present or by the rapidity with which it was respired or digested.

CROCKER and HARRINGTON (**16**), in studying the behavior of Johnson grass, found that storage of freshly harvested seed at 20° C. in the germinator led to an increased or secondary dormancy, a phenomenon frequently observed in seeds as a result of unfavorable germinating conditions. They suggest that such a deepened dormancy, if accompanied by a decreased respiration, may have an important bearing upon the longevity of seeds in the soil by lengthening the period necessary for the reduction of stored foods. From their own experiments on the respiration of Johnson grass they estimate the possible longevity of this seed as follows:

> If 75 per cent of the weight of the seed can be respired before death occurs, secondarily dormant Johnson grass seeds could lie in a germinator for 9.8 years at 20° C. before death would occur from exhaustion of stored foods. The period at 10° C. would likely be 2 to 3 times 9.8 years, in accord with the temperature quotient for respiration. Without such a reduction in respiratory intensity the possible longevity would be a little more than one-third as great, figured on the initial rate in the active seeds. Even if the longevity of imbibed seeds in the soil be dependent upon some contingent other than

exhaustion of stored food, this reduction in respiration is of significance. It will leave more stored material for building purposes in case germination does occur after a considerable period in the soil.

A similar calculation has been made of the rapidity with which *Amaranthus* seeds respiring at the rate observed (table IV) would exhaust their storage substance. The estimate is based on a moisture content of 47.43 per cent, and Woo's (44) analysis showing 47 per cent starch. On this basis the possible longevity is 160 days at the experimental temperature, 20°–25° C. This temperature is high, and respiration of stored food would certainly proceed more slowly at the lower temperature of the soil. Moreover, since observation (7, 8, 24, 41) shows the actual longevity of *Amaranthus* in the soil to be more than thirty years, there must be tremendous curtailment of metabolism under these conditions, with exceedingly slow use of the reserve. Even in the laboratory, dry-stored at room temperature and imbibed just before using, the seeds were viable 176 days after harvesting, and CROCKER reports that 200 days in the germinator at 20° C. does not alter their viability. If the 47 per cent fat contained in hawthorn be taken as stearin, the longevity of this seed when removed from the carpel, with 60 per cent water content, would be about 170 days at the rate of respiration observed (table IV) for the same temperature. Actually the seeds are viable for a longer time.

In all the rosaceous seeds studied variability of respiratory values was marked. Since the value of the respiratory quotient is based upon the volumes of CO_2 eliminated and of O_2 absorbed, it may serve as a convenient index of this variability. The total range of the quotient values of the six rosaceous seeds is 0.31–1.14. The extremes for individual seeds are as follows:

Hawthorn	0.47–1.14
Peach	0.56–0.96
Apricot	0.31–0.80
Cherry	0.76–1.04
Sand cherry	0.75–1.05
Blue gage plum	0.57–1.02
Burbank plum	0.72–1.04

As shown in fig. 4, with the exception of a single experiment on apricot, the quotients for all the other rosaceous seeds fall within the range of those of hawthorn. The mean for hawthorn (0.774)

lies within 0.02 of the mean of the means (table IV) for the other seeds (0.756). The rosaceous seeds, therefore, exhibit a marked similarity to one another in their respiratory behavior. If it may safely be assumed, as has been the tendency, especially among animal physiologists, that the character of respiration and particularly of the respiratory quotient depends upon the kind of substance oxidized, such a similarity would be expected, since in all these seeds the storage substance is chiefly fat.

On the other hand, in *Amaranthus*, although fluctuations in the carbon dioxide elimination and the oxygen absorption occur, and that too not always in the same, but occasionally in opposing, directions, the respiratory quotient remains relatively stable throughout a period of 176 days. The contrast in the behavior of the Rosaceae and of *Amaranthus* may be due in part to the difference in storage material, since *Amaranthus* contains little fatty substance (**44**), but much starch. This latter substance constitutes the reserve in *Chenopodium* and in *Rumex* also. It is probable, however, that other factors are responsible for the extreme variability of the rosaceous quotients.

The embryo of *Amaranthus* is not dormant. "Any time after maturity naked embryos are capable of immediate growth" (**16**). The six rosaceous seeds have dormant embryos. This dormancy, however, is of unequal intensity in different parts of the embryo. DAVIS and ROSE (**17**) and ECKERSON (**19**) have emphasized the difference in development of cotyledons and of the hypocotyl in *Crataegus*. DAVIS (**18**) finds a similar situation in the peach. It is therefore reasonable to suppose that these two parts of the embryo, cotyledons and hypocotyl, differing as they do physiologically and chemically, may differ in their metabolic activity and specifically in their oxygen absorption and carbon dioxide elimination. These differences at times may counterbalance, or at times augment, each other; or it may be that now the intensity of the hypocotyl, now that of the cotyledons, may predominate and determine the metabolism characteristic of the seed as a whole.

An analogous situation is reported by MAIGE (**32**) for stamens. In general the respiratory intensity of the adult stamen is less than that of the young organ, but this decreased intensity is differently attained in different plants. In some there is a steady decrease

from youth to age, while in others there may be an increase to a maximum followed by decline to the adult intensity, or a fall to a minimum succeeded by a return to a rate slightly lower than that at the beginning. Study of the filament and anther separately reveals the fact that their respiratory intensities are distinctly different, the anther undergoing a sort of grand period of respiratory intensity, while the intensity of the filament increases regularly from immaturity to maturity. The intensity of respiration of the stamen as a whole therefore is the resultant between these two respiratory intensities.

Great diversity of opinion exists as to the importance attaching to the respiratory quotient as an index of metabolism. In seeds like *Amaranthus* and *Chenopodium* the quotient would appear to be of significance because of its stability. The variability of the quotient in Rosaceae at first might appear to militate against its possessing any significance. When, however, this variability of the quotient is found to characterize a group possessing fundamental physiological and chemical features in common, it would seem that even here some significance might attach to the quotient. It may be of little value as indicating the material oxidized, but it may have considerable importance as indicating a situation due to the interplay of several factors. The quotient percentage curves (fig. 4) and the frequency histograms (fig. 3) show more clearly than do the tabulated data the general trend of respiration. From them can be seen that even with their variability the values for hawthorn and the other Rosaceae tend to fall into small groups about one largest assemblage. The latter, therefore, may be considered indicative of the type respiratory value for the seed.

In *Chenopodium* and *Amaranthus* the massing of the quotients is within a narrow range, and the type is more marked. Treatment of data in this way, therefore, may serve as a further check on the uniformity of conditions under which the experiments are carried out, and perhaps on the reliability of the method.

In this connection it is interesting to note that the curves in fig. 3 are of the kind found by PEARSON to be typical for botanical measurements, limited skew curves ("axis of the abcissas limited

on both sides, curve unsymmetrical"). Zoological curves, on the contrary, are unlimited skew curves ("axis of abcissas unlimited on both sides, curve unsymmetrical," **45**). A possible explanation of this difference in behavior between plants and animals that suggests itself is the complication of the results of zoological experimentation due to the independent volition of the animal. Plants, placed under a given set of conditions, vary little in behavior, while uniformity of behavior in the case of different animals, or even in the case of the same animal upon successive occasions, is beyond control.

These respiratory studies in no wise answer the queries that they suggest. They are rather preliminary to further investigation. Upon one point, the difference in respiration between dormant and after-ripened but still resting *Crataegus* seeds, some data have already been obtained. That the respiration is slightly higher in the after-ripened than in the dormant seed seems well established. Further study of this point, however, is necessary.

Summary

1. The respiratory intensity, that is, the mg. CO_2 eliminated per gram imbibed seeds per hour, was determined experimentally for *Amaranthus retroflexus*, *Chenopodium album*, and *Rumex crispus*, as well as for *Crataegus* and certain drupaceous Rosaceae. Determinations of the catalase activity were also made for most of the seeds.

2. Catalase activity increases in *Crataegus* under after-ripening and germinating conditions (10° C.), up to the forty-second day. The slightly higher value for the 128th day may represent: (1) a continued increase at an extremely slow rate; (2) a limit depending on the amount of dioxogen used (5 cc.); (3) a falling off, as a result of secondary dormancy, of an activity whose maximum occurred at the completion of after-ripening (about the ninetieth day). Respiration reaches a maximum intensity much earlier (sixth to eighth day), and thereafter exhibits a slow and fluctuating decline, at least to the seventy-seventh day.

3. In *Amaranthus* both catalase activity and respiration are relatively stable. Fluctuations in catalase activity and in respiratory

intensity do not occur simultaneously, and may be in opposite directions.

4. The respiratory quotient and respiratory intensity vary markedly for different seeds, and in the Rosaceae for different lots of the same kind of seed under precisely similar experimental conditions. The respiratory quotient in *Amaranthus* and *Chenopodium* is markedly stable. Since in the Rosaceae the embryo is dormant, while in the other two seeds it is not, it may be that this difference in behavior is characteristic of seeds with dormant embryos, and the greater stability of respiration in *Amaranthus* and in *Chenopodium* represents the attainment of a more stable metabolism in these seeds.

5. Stability or variability of the quotient may be of significance as indicative of the possibility of an interplay of several factors on the metabolism. In *Crataegus*, and presumably in the other Rosaceae, the marked variability is probably the resultant between the respiration of the dormant hypocotyl and that of the mature cotyledons.

6. The arrangement of the respiratory quotients for each seed in a curve showing the percentages of the experiments with each seed giving each value, and in frequency histograms in which are plotted the actual number of experiments in which each quotient value occurred, indicates the tendency of each seed toward a typical respiration. The quotients for *Chenopodium* and *Amaranthus* are 0.928 and 0.856 respectively, while those of the Rosaceae form three groups within a range of 0.118. In the first group, between 0.648 and 0.7, fall the quotients for apricot, peach, and blue gage plum; in the third, between 0.8 and 0.876, those of cherry and sand-cherry; while that of hawthorn, 0.774, lies midway between.

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LEAVES OF THE HELOBIEAE[1]

AGNES ARBER

(WITH PLATE I)

Introduction

In a paper published in 1918 (1), the phyllode theory of the monocotyledonous leaf was discussed in general terms, and in subsequent articles in this and other journals (**2, 3, 4, 5**) attempts have been made to trace the results of applying this theory in various special cases. In the present paper, it is proposed to study the leaves of the Helobieae, to see how far the phyllode theory will help toward interpreting them. I am indebted for material to Professor OSTENFELD of Copenhagen; to the Director of the Royal Botanic Gardens, Kew; to the Keeper of the Department of Botany, British Museum (Natural History); and to the Superintendent of the Cambridge Botanic Garden.

The Helobieae of ENGLER consist of seven families of water or marsh plants. Their common characters are difficult to define, but they are united by a macropodous embryo, and on the whole they appear to form a fairly coherent group. The seven families will be considered individually, and then the general conclusions drawn. Since I believe that the Alismaceae and their allies include the less specialized types within the cohort, these families will be discussed first, instead of following ENGLER's sequence.

Alismaceae

The literature on the protean leaf forms of the Alismaceae has been summarized elsewhere (**6**). The point to emphasize now is that the leaves of this family fall into three categories.

1. *Leaves with a sheathing base and a limb, more or less radial in form and phyllodic in anatomy.*—This form of leaf is rare in the family, but is found in the Sagittarias of the *S. teres* group, to which *S. isoetiformis* Smith and *S. teres* Watson belong. Fig. 1

[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher–Warr Studentship of the University of London.

represents a transverse section of the awl-like limb of one of these peculiar American Sagittarias. For comparison, by its side is a transverse section of the petiole of the normal arrowhead leaf of *S. sagittifolia* L. It will be recognized at once that both in form and structure they are essentially identical, and it will probably be generally agreed that the leaf of *S. teres* is equivalent to the arrowhead leaf of *S. sagittifolia*, minus the blade. DOMIN (9) evidently takes this view, for he uses the term "Phyllodien" in describing these leaves.

2. *Leaves with a sheathing base and a flat ribbon-like limb.*—These leaves are exceedingly common in the family, and are regarded as equivalent in morphological value to type 1, since in *S. sagittifolia* intermediate forms can be traced between thin ribbon leaves with a single row of bundles (fig. 3) and the almost radial petioles of the arrowhead type (fig. 2). For instance, among the transitional leaves between the juvenile ribbon and the mature arrowhead, a leaf was examined which was ribbon-like, but with a spathulate apex. It was found that the ribbon region was thicker than in the simple ribbon leaf, and, instead of having one series of bundles only, it had one small additional bundle above and one below the median bundle, and one below each of the main laterals, that above the median bundle being inverted. This showed an approach to the radial structure of the *S. teres* group.

3. *Leaves with a differentiated pseudo-lamina.*—I have set forth elsewhere (1) the reasons for regarding the blade of such leaves as the arrowhead as "pseudo-laminae," produced by the expansion of the distal part of the petiolar phyllode. How far the form and venation of the blades of the Alismaceae harmonize with this interpretation may now be considered. In some of the oval or cordate leaf forms there is little difficulty in seeing how the skeleton of the blade might be produced merely by the separation of the parallel petiolar veins, which at the apex return to their original approximation. This is the case, for instance, in *Alisma parnassiolium* Bassi var. *majus* (fig. 6). A further development on the same lines has taken place in *A. nymphaeifolium* Griseb. (fig. 8), in which the veins *v* and *v'*, curving into the basal lobes, give off second-

ary veins, more or less parallel to themselves, and thus, without any essentially fresh departure, achieve a venation determining the auricled form of the leaf. It is probable, however, that such leaves do not form a transition to the arrowhead type, but that the latter is arrived at by a separate route.

It will be seen on examining fig. 5 (*Limnophyton obtusifolium*) and fig. 9 (*Sagittaria Greggii*) that the principal veins are the midrib (*a*) and the two veins (*b*, *b'*) passing into the cusps. In some species these cusps are very conspicuous; in *S. longiloba* they may be two or three times the length of the median segment. It is not probable that the arrowhead type of venation is derivable from that shown in fig. 8, which is, moreover, a rarity in the family. It is suggested that the veins *b* and *b'* are, as it were, a repetition of the midrib, and have originated phylogenetically by its chorisis. Their morphological relation to the midrib would thus be equivalent to the relation borne by the tendrils of *Smilax* to the petiole, according to a hypothesis put forward in a previous paper in this journal (**4**). Of course it is impossible to offer any definite proof of such a theory, but it probably makes the nature of the arrowhead leaf a shade less obscure. It seems to account for the lack of any genuinely transitional forms between the types of venation characterizing the oval and arrowhead varieties of pseudo-lamina. It is true that the intermediate forms have very short cusps, but their venation is distinctly of the arrowhead type.

Butomaceae

The Butomaceae are so closely related to the Alismaceae that they are sometimes regarded merely as a tribe of the latter family. Among the Butomaceae we find examples of the three types of leaf enumerated under the Alismaceae. *Butomus umbellatus* L. has a leaf with a sheathing base, and an upper region which is triangular in section and phyllodic in anatomy (**17**). On the other hand, *Hydrocleis Commersonii* Rich. and *H. parviflora* Seub. have both ribbon leaves and leaves with a petiole and pseudolamina (**10, 18, 20**). The published figures of these ribbon leaves suggest that they are equivalent to the ribbon leaves of *Sagittaria*, but I have not had the opportunity of examining their anatomy.

Juncaginaceae

Of the five genera of Juncaginaceae, none possesses leaves with pseudo-laminae. Certain species of *Triglochin* have ribbon-like leaves, for example, *T. montevidense* Spreng., figured by SEUBERT (**18,** pl. XII), and *T. procerum* R.Br. It is hoped to deal with the leaf anatomy of this genus in a later paper. Except in the case of these few ribbon-leaved species, all the five genera have leaves with a sheathing base and a radial or ensiform limb, with a typically phyllodic anatomy. In *Triglochin*, *Lilaea*, and *Scheuchzeria*, a ligule generally marks the boundary between sheath and limb (**9**). Fig. 7 shows the transverse section of the limb of the leaf of *Scheuchzeria palustris* L. Its curious asymmetry has been figured and commented on by RAUNKIAER (**15**). The leaf of the monotypic *Maundia*, judging from BUCHENAU'S figure of the transverse section (**8,** fig. 7, p. xvi), is phyllodic, and similar to that of *Triglochin maritimum* (**1**). I have cut the leaf of the monotypic *Lilaea subulata* Humb. et Bonpl., and, although the herbarium material available was not very favorable for anatomical work, it was obvious that here also the structure is phyllodic. The leaf, which is described as awl-shaped and cylindrical in the fresh condition, is supplied by a series of small peripheral bundles in addition to three main strands. HIERONYMUS (**11**), in a Spanish monograph of *Lilaea* published forty years ago, definitely states that the leaves of this plant consist only of sheath and petiole, the lamina being unrepresented.

It was interesting to find, on examining the fifth genus (the monotypic *Tetroncium magellanicum* Willd.) that the leaf is unusual among the Helobieae in being of the ensiform type, and in having a bundle system identical with that of the isobilateral equitant leaves which are so familiar, for instance, in *Iris*. In fig. 4, *A* shows the structure of the sheath region, *B* the transition to the limb, and *C* the limb itself, which has a close anatomical resemblance to that of *Tofieldia calyculata* Wahl., one of the equitant members of the Liliaceae (**1**). Fibrous sheaths are associated with the bundles.

Potamogetonaceae

In this family there are three types of leaf, corresponding to those met with in the Alismaceae and Butomaceae. The rarest

type is the leaf with a sheathing base and more or less radial limb with phyllodic anatomy. This leaf form is found in *Cymodocea isoetifolia* Aschers. (fig. 11), described by SAUVAGEAU (**16**) and OSTENFELD (**14**). A ring of bundles surrounds the median strand. The same genus also includes leaves in which the limb is flat and furnished with only one series of bundles (as *C. nodosa* Aschers., fig. 12), while *C. manatorum* Aschers. (fig. 10), with its more or less terete leaf, traversed by three strands only, forms an intermediate type. In each species there is a ligule, clearly delimiting the sheath from the petiolar region.

In *Potamogeton* the leaves of some species, like the air leaves of many Alismaceae, possess pseudo-laminae. In *P. natans* L. there are, in addition, bladeless, terete, phyllodic leaves, corresponding exactly in structure and anatomy to the petioles of the fully developed leaves (**16**), and which may be regarded as equivalent to the leaves of *Cymodocea isoetifolia*. *P. natans* also has leaves showing a further degree of reduction, but instead of being ribbon-like, as in the Alismaceae, they consist almost entirely of the highly developed axillary stipules or ligules.

Naiadaceae

The leaves of the Naiadaceae are much reduced, but they have a sheath and a thin flat limb, and thus correspond to the ribbon leaves of *Sagittaria*.

Aponogetonaceae

Only the one genus, *Aponogeton*, is included in the Aponogetonaceae. Most of the species have leaves with a differentiated sheath, petiole, and pseudo-lamina, but *A. vallisnerioides* Bak. has ligulate ribbon leaves, described (**7**) as resembling those of *Vallisneria*, while in *A. spathaceum* E. Mey. var. *junceum* Hook. f. (**12**) semiterete leaves with a sheathing base are found. A piece of a leaf of this variety which was examined did not include the distal part of the leaf, but showed the structure of the transition region between sheath and limb. In addition to the three main bundles, which HOOKER indicates in a slightly magnified transverse section which he figures, there are a number of small peripheral bundles. The structure is thus closely equivalent to that of the petiole of such a species as *Aponogeton distachyum* Thunb.

Hydrocharitaceae

In the Hydrocharitaceae all three types of leaf which have been considered are found. In *Stratiotes* and *Enhalus* there is no blade, and the occurrence of inverted bundles (**13, 19**) gives a phyllodic aspect to the anatomy. These leaves may be regarded as equivalent to that of *Butomus*, but in *Stratiotes* there is no sheath, although this region is developed in *Enhalus*. *Hydrocharis* has a leaf with a stipulate sheath, a petiole, and a pseudo-lamina, while the leaves of *Vallisneria* and *Thalassia* are similar to the ribbon leaves of *Sagittaria*. *Vallisneria* has a sheath which may easily be overlooked, while in *Elodea* this region is entirely lacking (**9**).

Conclusions

A comparison of the leaf structure in the various families belonging to the Helobieae shows the repeated occurrence of that leaf type in which there is a sheathing base succeeded by a bladeless limb, in appearance and structure recalling a petiole. Such leaves, instances of which are met with in six of the seven families, are regarded as *typical petiolar phyllodes*. This simple phyllodic form of leaf is most characteristically developed in the Juncaginaceae, where it occurs in all five genera. The leaves in this family are generally more or less radial, except in *Tetroncium*, where they are ensiform and *Iris*-like. In the other families leaves of approximately radial structure occur more or less sporadically, or as rarities, except in the Naiadaceae, where they are entirely lacking. The extreme reduction of the one genus *Naias*, which constitutes the family, however, makes the absence of such leaves by no means surprising.

In each of the seven families examples of a leaf with a sheathing base and flat ribbon-like limb are found. This leaf is regarded as equivalent to the more obviously phyllodic type of leaf just discussed. Two lines of evidence point to this conclusion: (1) within the single species *Sagittaria sagittifolia* L. transitions, both in external form and internal structure, can be found between typical ribbon leaves and petioles; and (2) within *Cymodocea* not only typical ribbon leaves (as *C. nodosa*, fig. 12) and typical petiolar leaves (as *C. isoetifolia*, fig. 11) are found, but also in *C. manatorum* (fig. 10) there is an intermediate link between these two types.

The third and last leaf type, that in which there is a differentiated blade, occurs in five of the seven families, the exceptions being the Juncaginaceae and Naiadaceae. It is not necessary here to discuss the evidence, based partly on a study of the succession of leaf forms in the ontogeny, and partly anatomical, which has led to the conclusion that these blades are "pseudo-laminae," originating by the expansion of the apex of the petiolar phyllode, for this question has been considered elsewhere (**1, 6**). The present paper adds a study of the significance of the blade venation of the Alismaceae.

The final impression left by this survey of the leaves of the Helobieae is that there has been a remarkable parallelism of development within the different families. The three leaf types enumerated recur throughout the cohort in forms which, although modified in various ways, are identical in essentials.

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EXPLANATION OF PLATE I

The plate shows the xylem in black, the phloem in white, the fibers (*f*) dotted, and the outlines of lacunae in dotted lines.

FIG. 1.—*Sagittaria* of *S. teres* group: transverse section of limb of leaf *f*, interrupted fibrous sheath of bundle (slight asymmetry of section probably due to incomplete recovery of herbarium material used; Georgia Plants. Roland Harper. 1473. Ex Herb. Brit. Mus.); ×23.

FIG. 2.—*Sagittaria sagittifolia* L.: transverse section of petiole close to blade, fibrous bundle sheath (less highly developed than in species shown in fig. 1) not indicated; ×14.

FIG. 3.—*Sagittaria sagittifolia* L.: transverse section of small ribbon leaf, ×23.

FIG. 4.—*Tetroncium magellanicum* Willd.: transverse sections of leaf; *A*, sheath, *B*, transition to limb; *C*, limb; ×23.

FIG. 5.—*Limnophyton obtusifolium* Miq.: blade of leaf; *a*, midrib; *b*, *b'* cusp veins (serration of margin probably an effect of drying); ×0.5.

FIG. 6.—*Alisma parnassifolium* Bassi var. *majus*, blade of leaf; ×0.5.

FIG. 7.—*Scheuchzeria palustris* L.: transverse section of limb of leaf; *f*, fibrous strand occupying one margin (on account of small scale, fibrous sheaths of bundles not separately indicated); ×23.

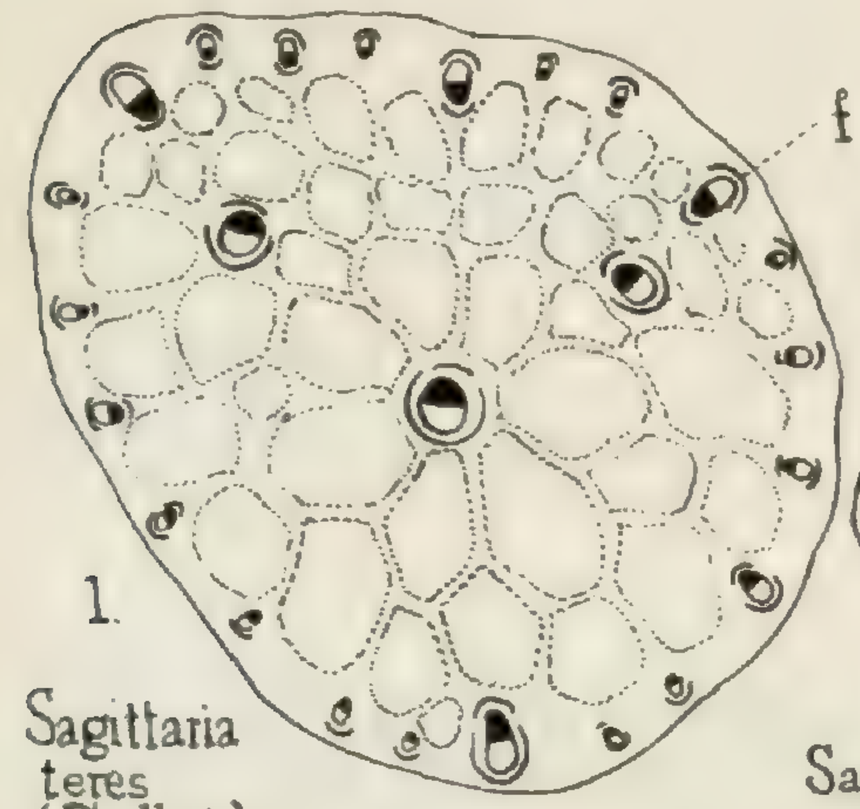
FIG. 8.—*Alisma nymphaeifolium* Griseb.: blade of leaf; *v*, *v'*, principal veins of auricles; ×0.5.

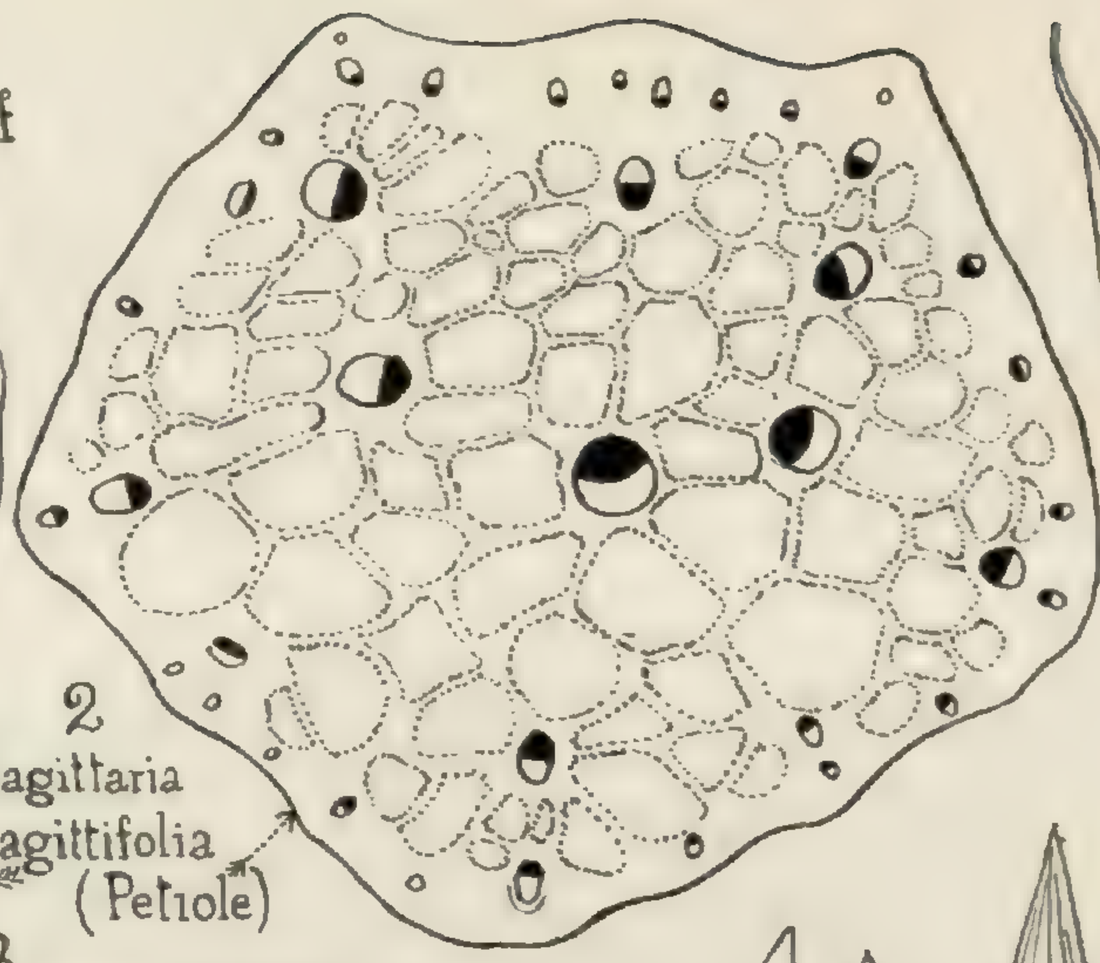
FIG. 9.—*Sagittaria Greggii* Smith.: blade of leaf; *a*, midrib; *b*, *b'*, cusp veins; ×0.5.

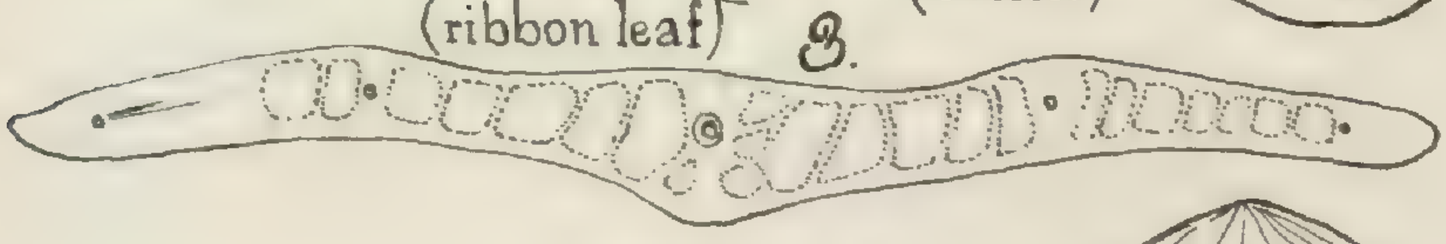
FIG. 10.—*Cymodocea manatorum* Aschers.: transverse section of limb of leaf; ×23.

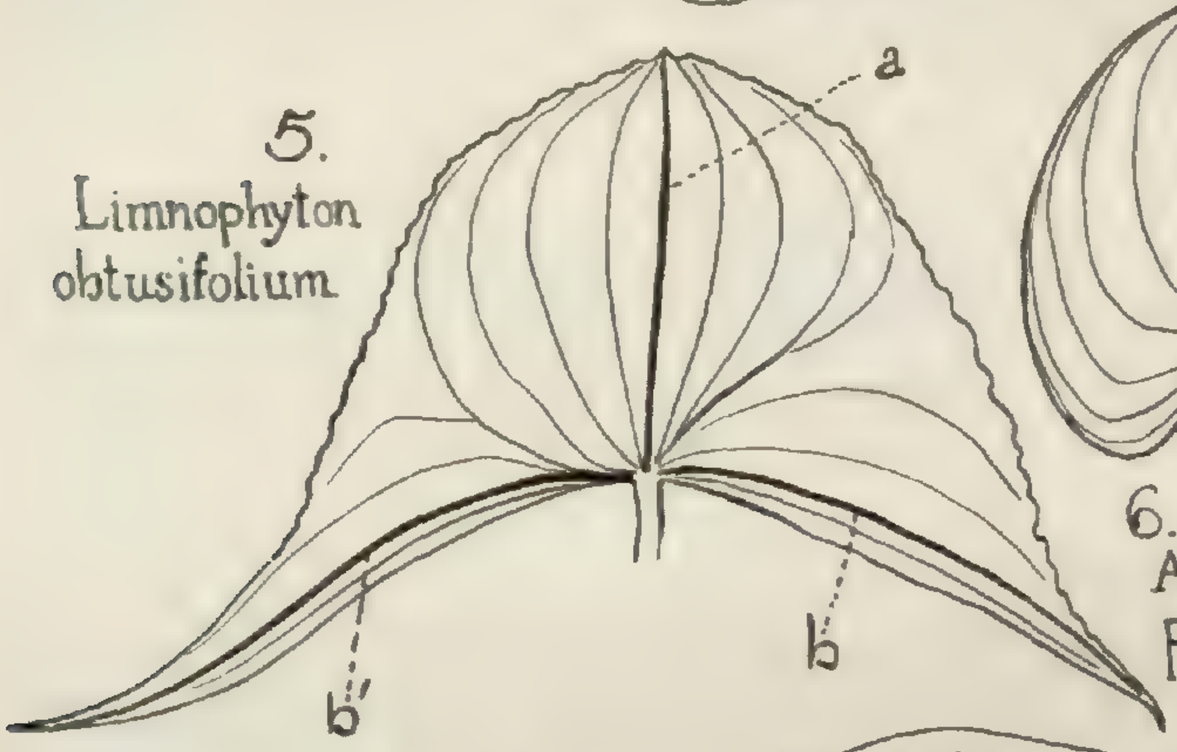
FIG. 11.—*Cymodocea isoetifolia* Aschers.: transverse section of limb of leaf; xylem indistinguishable, in smaller bundles surrounding median bundle; ×23.

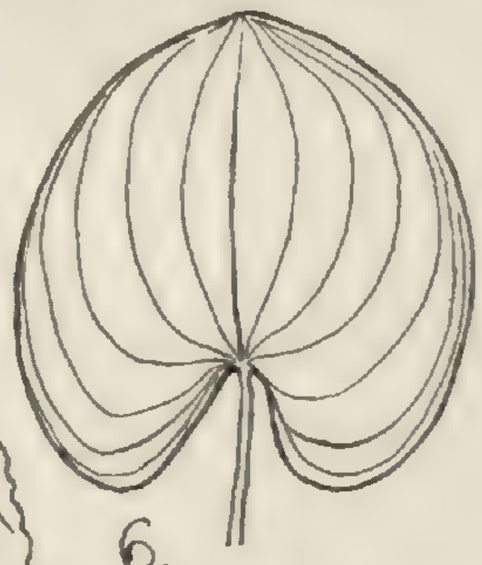
FIG. 12.—*Cymodocea nodosa* Aschers.: transverse section of limb of leaf; ×23.

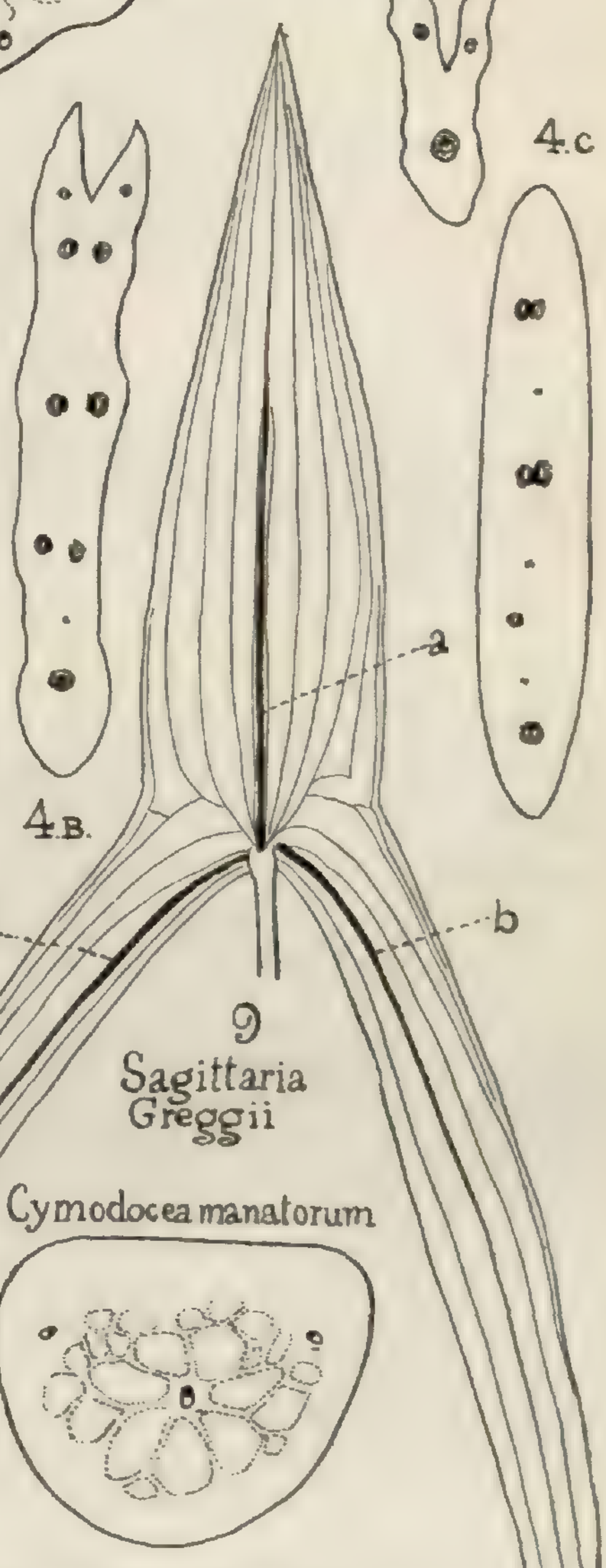


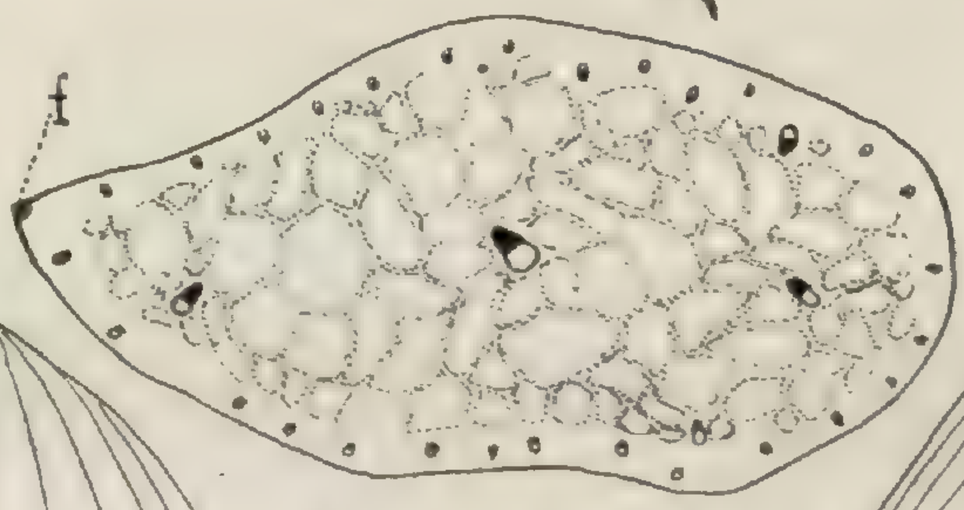


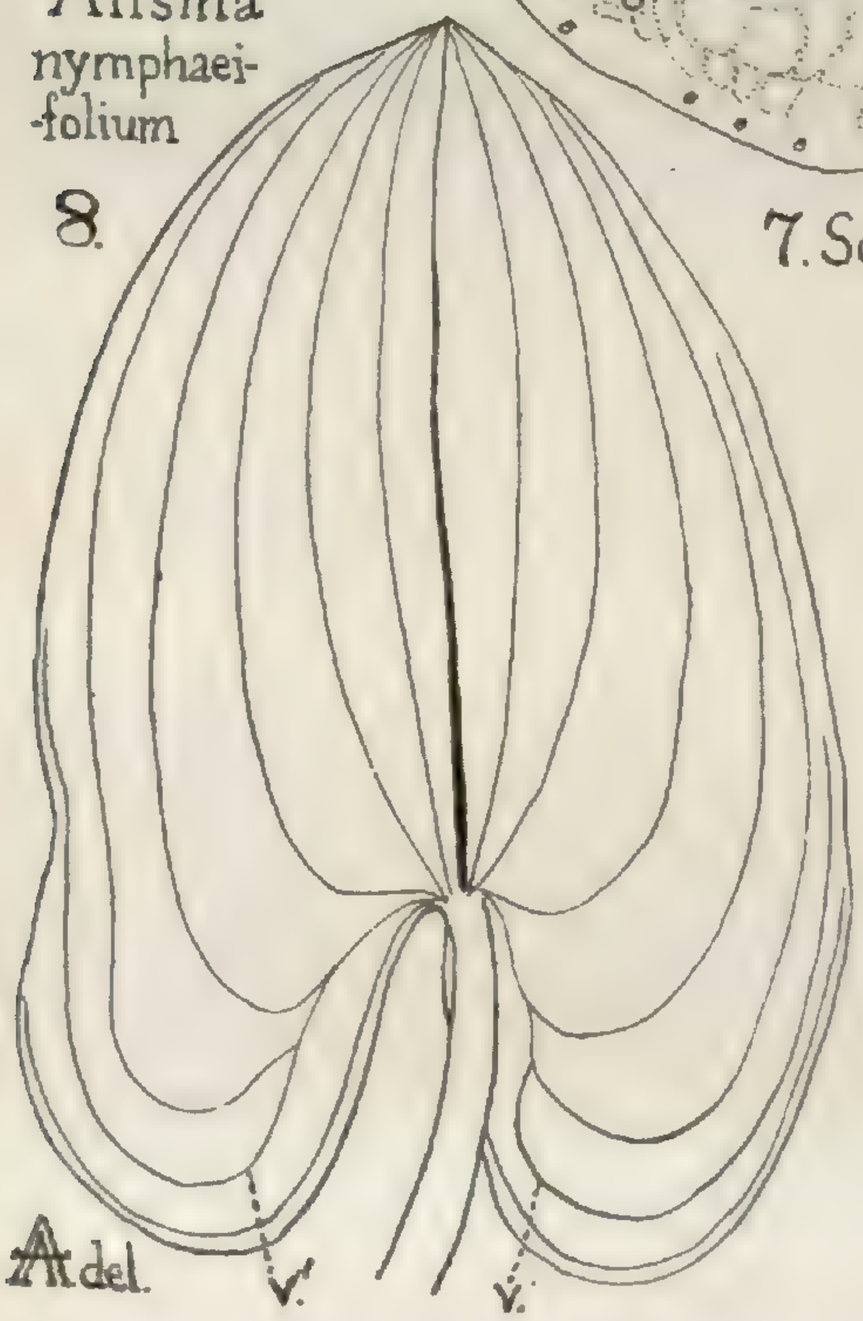


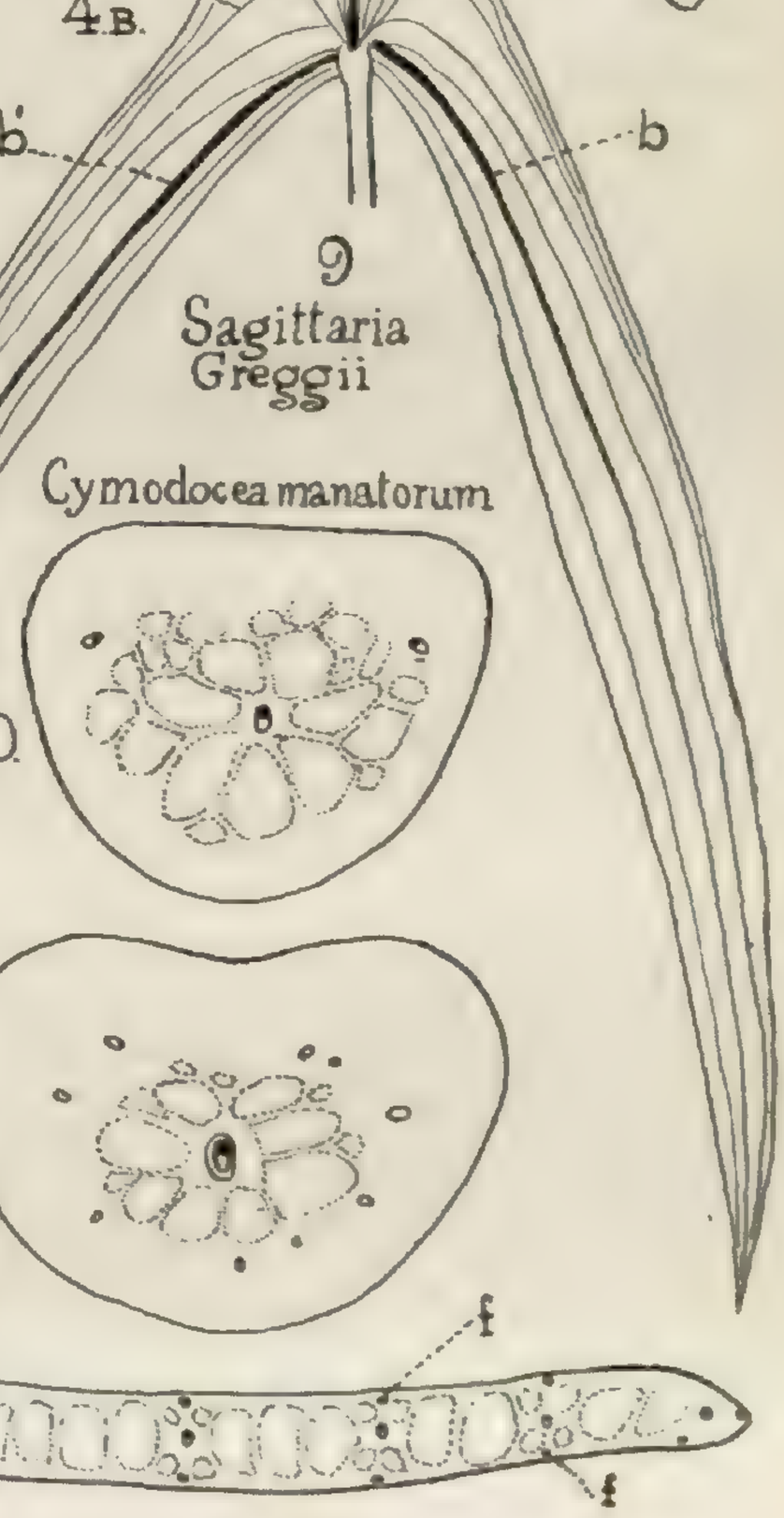












ARBER on HELOBIEAE

NOTES ON NEW OR RARE SPECIES OF RUSTS

W. H. Long

This paper describes four new species of rusts, namely, *Gymnosporangium cupressi* on *Cupressus arizonica*, *Ravenelia subtortuosae* on *Acacia subtortuosa*, *Ravenelia gooddingii* on *Acacia suffrutescens*, and *Ravenelia cassiae-covesii* on *Cassia covesii*, and gives new data as to hosts and distribution of two other species of *Ravenelia*.

Gymnosporangium cupressi Long and Goodding, sp. nov.

I. Aecia unknown.

III. Telia caulicolous, from a perennial mycelium, appearing on twigs, branches, and trunks, causing fusiform to subglobose swellings 1–90 cm. long by 0.5–30 cm. thick, usually breaking forth irregularly and often transversely on the smaller branches and twigs, in irregular rows in deep longitudinal fissures of the bark on the larger branches and trunks. When mature, telia are more or less wedge-shaped, often irregular and somewhat crenate at top, before gelatinization 2–10 mm. broad by 4–6 mm. tall, dark chestnut brown, becoming cinnamon brown after expansion; teliospores 2-celled, spores with colored walls, oval to ellipsoid, 22–27×43–50 μ, average for ten spores 24.2×49 μ, slightly or not at all constricted at the septum, the two cells subequal, pedicel cylindrical, pores two in each cell near septum, walls 2–3 μ thick; teliospores with thin, colorless walls, oblong to narrowly ellipsoid, not constricted at septum, 16–20×40–60 μ, average for ten spores 18.6×53 μ, the two cells subequal, spores rounded at both ends, pores two in each cell at the septum, walls 1–1.5 μ thick.

On Juniperaceae. Type collected on *Cupressus arizonica*, at Snebly Hill, 3.5 miles from Sedona, Arizona, May 26, 1920, by *Leslie N. Goodding* (no. 6906 Long); also collected on same host and in same locality in 1919 by *Goodding* (no. 6903 Long). Collected on same host on road between Cottonwood and Sedona, 6 miles from Sedona, May 26, 1920, by *Goodding* (no. 6904 Long). This fine species of *Gymnosporangium* is probably generally distributed on this host in the draws and canyons around Sedona at an elevation of about 4000 ft.

Ravenelia subtortuosae, sp. nov.

0. Pycnia; none found in material at hand.

I. Aecia caulicolous, thickly scattered over hypertrophied areas which form open witches' brooms 1–2 cm. across. Aecia 0.2–0.3 mm. in diameter by 0.8–1.2 mm. high, cylindrical, subepidermal, peridium erect, margin erose and gradually weathering away to base, cells irregularly oblong to polygonal in face view, not overlapping, outer walls 5–6 μ thick, verrucose, inner ones 2–3 μ thick, verrucose, both walls appearing as if reticulate in certain views, side walls 2–3 μ thick, transversely striate; aeciospores irregularly oval to subglobose, angular, 13–18×18–23 μ, average for ten spores 17×19.6 μ, walls cinnamon brown, 2–3 μ thick, minutely verrucose.

II. Uredinia amphigenous, very small, less than 0.3 mm. across, rather firm, punctiform, subepidermal, ruptured epidermis inconspicuous; urediniospores obovate, subpyriform to oval, 15–22×22–30 μ, average for twenty spores 17.3×25.7 μ, chestnut brown, concolorous, or sometimes slightly darker at apex, walls 1.5–2 μ thick, uniform, verrucose, germ pores six, equatorial; paraphyses very abundant, often constituting one-third to one-half of sorus, hyphoid, incurved, chocolate brown, dense, encircling the sorus, 10–13×40–50 μ, average for ten 10.3×43.6 μ, an occasional paraphysis clavate, nearly colorless and with a solid stipe.

III. Telia amphigenous, oval, 0.5–1 mm. across, chestnut brown, subepidermal, ruptured epidermis inconspicuous, early naked; paraphyses none; teliospore heads light brown, hemispherical to ovoid, very irregular in shape and size, 33–100 μ, average for forty heads 52.5 μ, 3–6 spores across, marginal spores 3–16, inner spores 0–12, spores in head 3–32, usual number 14–25, smooth, outer spores 1-celled, inner ones 2-celled; cysts small, hyaline, subappressed, ovoid, as many as the marginal spores, cohering at sides to each other but not to stipe, swelling and bursting in water; pedicel hyaline, compound, deciduous, short, 32–55 μ long.

On Mimosaceae. Type for aecia collected on *Acacia subtortuosa* at Corpus Christi, Texas, May 25, 1918, by *W. H. Long* (no. 6506). Type for

uredinia and telia collected on same host and in same locality June 25, 1920, by *Long* (no. 6891); also collected at Darling, Texas, on same host June 19, 1920, by *Long* (no. 6892).

The aecial stage of this *Ravenelia* is very conspicuous, while the other two stages are just the reverse. In fact, to find the uredinia and telia one must look on the leaves immediately adjacent to the aecial stage. At Darling, a flag station about 12 miles from Spofford Junction, the old witches' brooms of this *Ravenelia* were very abundant for about 1 mile along the railroad track. Often some bushes would have from fifteen to twenty-five "brooms." An occasional urediniospore was found intermixed with the telia in the Darling material.

In the 1918 collection aecia and telia were found. The telia were very rare, only a few sori to each bush. In the 1920 material collected in the same catclaw-mesquit field, an abundance of both uredinia and telia were found associated directly with the old witches' brooms. The uredinia were found on bushes growing in low damp spots with branches dense and close to the ground. On account of the abundance of the uredinia and telia in the 1920 material this collection was made the type of these two stages.

The uredinial stage of *Ravenelia subtortuosae* bears a close resemblance in all of its characters to the same stage of *R. australis* (as it occurs in Texas), even to the paraphyses, but differs materially in its telia from this species. *R. subtortuosae* is also related to *R. MacOwaniana*, found in south and central Africa on *Acacia horrida*, but differs from this species in many important characters.

This is the only *Ravenelia* known to the writer reported from the Americas that has the three stages, aecia, uredinia, and telia.

Ravenelia gooddingii, sp. nov.

o. Pycnia unknown.

II. Uredinia small, sparse (in material examined), hypophyllous, scattered, subcuticular, early naked, cinnamon brown; paraphyses very abundant, intermixed with the spores or in separate sori, subcylindrical to narrowly clavate, a few obovate, clavate type with thick walls and nearly solid heads, upper one-half to two-thirds of head fulvous, balance hyaline or nearly so, stipe solid, hyaline, 10–14 by 40–50 μ, obovate type thin-walled, subhyaline, with apex sometimes slightly thickened and fulvous, 15–18 × 30–55 μ; urediniospores broadly oval to globoid, 12–16 × 16–19 μ, walls pale fulvous, thin, 1–1.5 μ, verruculose, pores 6–8, scattered.

III. Telia amphigenous, but mainly hypophyllous, often seated on pallid spots, usually found on basal half of the leaves, very irregular, 0.5–1.5 mm. × 2–4 mm. long, often confluent over one-half to two-thirds of the leaf, subcuticular, early naked, shining,

chocolate brown, ruptured cuticle inconspicuous; paraphyses none, teliospore heads light chestnut brown, 5–6 cells across, 60–80 μ, average for twenty heads 70.3 μ, 8–16 marginal cells, 8–18 inner ones, heads more or less flattened, smooth; cysts hyaline, in two rows beneath the entire head, appressed, not cohering, oval to obovate, easily swelling and bursting in water; pedicel short, hyaline, deciduous.

On Mimosaceae. Type collected on *Acacia suffrutescens* in Baboquivari Mountains, Arizona, October 24, 1919, by *Leslie N. Goodding* (no. 6983 Long).

Ravenelia cassiae-covesii Long and Goodding, sp. nov.

0. Pycnia unknown.

II. Uredinia amphigenous, scattered, round or irregular, 1–2 mm. across, subcuticular, early naked, cinnamon brown, ruptured cuticle inconspicuous; paraphyses very few, intermixed with the spores, clavate-capitate to capitate, hyaline, 11–13 × 37–66 μ, average for ten paraphyses 12.5 × 48 μ, stipe solid, hyaline, about 5 μ thick, walls of heads thin, 1–1.5 μ thick, smooth; urediniospores broadly ellipsoid, obovate to subglobose, 15–20 × 17–23 μ, average for forty spores 17.2 × 20.1 μ, walls cinnamon brown, 2–2.5 μ thick, verrucose-echinulate, germ pores eight, in two irregular zones of four pores each, equidistant from the equator, or scattered in the subglobose type.

III. Telia amphigenous and caulicolous, scattered, round, 0.5–1 mm. across, subcuticular, early naked, chocolate brown, ruptured cuticle inconspicuous; paraphyses few, similar to those found in the uredinia; teliospore heads chocolate brown, 5–7 cells across, 50–84 μ, average for forty heads 65.3 μ, 6–14 marginal cells, 6–20 inner ones, heads smooth or with half to two-thirds of the cells bearing a single, wartlike, semihyaline papilla, 1–4 μ long, to each cell; cysts numerous, hyaline, globose, subappressed, in two or three rows, beneath entire head, slowly swelling and bursting in water; pedicel short, hyaline, deciduous.

On Caesalpiniaceae. Type collected on *Cassia covesii* near Tucson, Arizona, by *H. W. Thurston* and *Leslie N. Goodding*, February 26, 1920 (no. 5537 Long); also collected on same host in Sabino Canyon, near Tucson, March 9, 1920, and January 4, 1921, by *Leslie N. Goodding* (nos. 6918 and 6972 Long).

This species is intermediate between *Ravenelia mesillana* and *R. papillifera*. Some of the mounts from the material collected near Tucson (no. 5537) have nearly all of the teliospore heads smooth, while other slides from the same locality, as well as the material collected in Sabino Canyon (no. 6918 Long) in the foothills of the Santa Catalina Mountains, have a large number of the heads papillate.

Ravenelia siliquae Long

This rust was collected June 1920, at San Antonio, Texas, on the leaves, twigs, branches, and pods of *Acacia farnesiana*. In many cases young pods were found with the uredinia just sporulating, while the leaves and twigs of the same tree showed old uredinia. This proves the writer's contention in a previous article[1] that the twig and leaf rust on this host was *R. siliquae*, which up to that time had only been collected on the pods.

Ravenelia fragrans Long

A *Ravenelia* collected on the leaves and pods of *Mimosa biuncifera* in Arizona, by *Leslie N. Goodding*, was sent to the writer for identification. A careful comparison of this material with the type of *R. fragrans* shows no essential characters sufficient to warrant making it a new species. The paraphyses in the Arizona rust are slightly more clavate than those found in the typical *R. fragrans*, while many of the teliospore heads are nearly smooth. Each of the papillate cells bears 1–4 hyaline papillae, 1–3 μ long. The stipe is usually short, hyaline, and deciduous, but occasionally one is found which measures up to 100 μ. Many telial heads of the typical *R. fragrans* show cells with few and very short papillae similar to the Arizona material.

This rust has been collected in two localities in Arizona, the Baboquivari Mountains, October 24, 1919 (nos. 6534 and 6535 Long), and Rosemond, December 17, 1920 (no. 6969 Long). The latter collection has a large percentage of the teliospore heads smooth or with only an occasional head showing any papillate cells, while the 1919 material has heads fairly typical of *R. fragrans* as it occurs on *Mimosa fragrans*, yet the two collections are unquestionably the same species.

[1] Notes on new or rare species of *Ravenelia*. Bot. Gaz. **64**:57–69. 1917.

On a recent trip (June 1920) through Texas many areas were revisited which in May and November of 1916 showed an abundance of *Ravenelia* infection of many different species, yet only occasionally was any *Ravenelia* found, although a careful search was made on hundreds of plants which in 1916 were literally covered with *Ravenelia* sori. The species of *Ravenelia* so abundant in 1916 were as follows: *R. siderocarpi*, *R. papillifera*, *R. roemerianae*, *R. mesillana*, *R. gracilis*, *R. leucaenae*, *R. siliquae*, and *Neoravenelia holwayi;* of these only *R. siliquae* was at all common in 1920. This would indicate that certain years are very favorable for the propagation and dissemination of species of *Ravenelia*. This fact, of course, is well known in connection with various species of grain rusts.

OFFICE OF INVESTIGATIONS FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
ALBUQUERQUE, N.M.

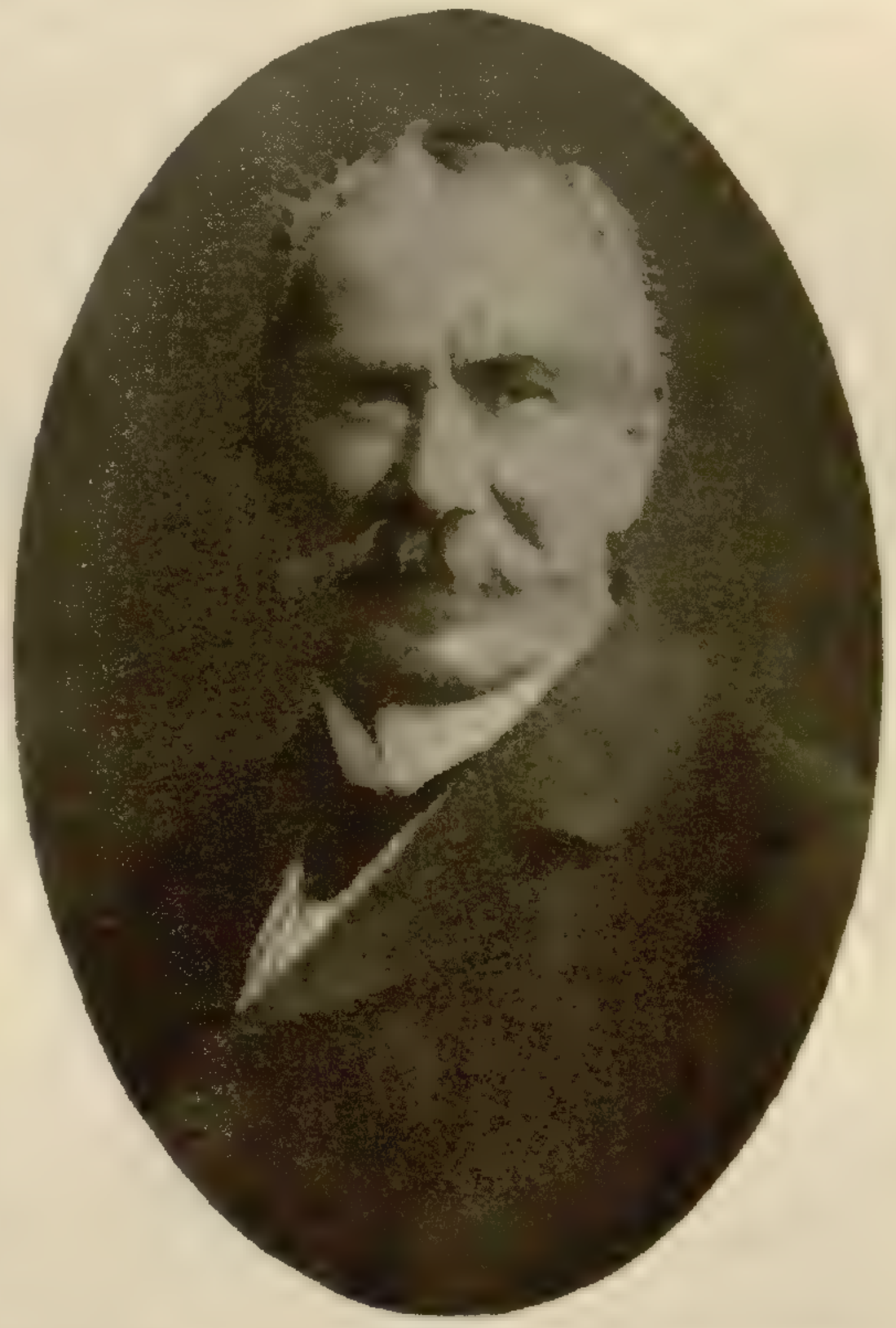
BRIEFER ARTICLES

HELMUT BRUCHMANN

(WITH PORTRAIT)

The name of BRUCHMANN has become so familiar through his indefatigable researches upon the prothallia of temperate species of *Lycopodium*, that some account of his life should appear in this journal, which has so often paid the last tribute of respect to great botanists.

HELMUT BRUCHMANN was born in Pomerania, Prussia, on November 13, 1847, and death came suddenly at Gotha on Christmas 1920. After the usual studies in local schools, he went to Jena, where STRASBURGER was beginning his great career as a teacher, investigator, and maker of investigators. Although I cannot find any authoritative data, it is my recollection that STRASBURGER himself told me that BRUCHMANN was the first man to take the Ph.D. degree under his direction, and that the thesis dealt with *Lycopodium*. STRASBURGER held him in the highest esteem and made him his assistant. Like STRASBURGER, he was a master of technique, making splendid sections before the days of paraffin and microtomes. In 1878, at the age of 29, he went to Tharand to deliver a course of lectures on forestry; and a year later was called to Gotha as teacher of mathematics and physics, and afterward biology, in the high school. STRASBURGER offered him an "ausserordentlich" professorship at Jena, but the stipend attached to that position at that time was so small that he felt compelled to remain at Gotha, with the comparatively comfortable salary of 2400 marks. Since BRUCHMANN married Fräulein EMMA JUSATZ in 1880, one might



surmise what prompted the decision. She shared, in an unusual degree, the cares and joys of his school life, and was intimately acquainted with his investigations and discoveries. For thirty years he taught mathematics and physics in the high school at Gotha, spending vacations and leisure hours in his patient and thorough investigations of *Lycopodium*. He was a successful teacher, reaching the highest rank in the school and often assuming the duties of Director. His thorough knowledge of his subject, together with a kindly, sympathetic disposition, won for him the respect and affection of his students. In 1905 his health became impaired, and he went to the Riviera to recuperate; but after several months, not feeling strong enough to resume the heavy burden of teaching, he retired upon a pension. In 1907, he visited Sicily, Tunis, and Algiers. In his later years, failing eyesight made the search for subterranean prothallia very difficult.

BRUCHMANN'S great contribution to science was his prolonged and successful investigation of the prothallia of the European species of *Lycopodium*. When he began his studies, nothing was known of the prothallia of temperate species except fragmentary accounts of the aerial prothallia of *L. inundatum* and the subterranean prothallia of *L. annotinum*. BRUCHMANN succeeded in finding practically complete series in the development of the prothallia of *L. complanatum*, *L. annotinum*, *L. clavatum*, and *L. Selago;* and his excellent histological technique and his skill as an artist, together with a clear literary style, enabled him to make an effective presentation of his researches and conclusions. Altogether there are 17 papers, but the most important are "Über die Prothallien und die Keimpflanzen mehrerer Europäischer Lycopodien," an extensive account with 199 pages and 8 plates, which appeared in 1898; and "Die Keimung der Sporen und die Entwickelung der Prothallien von *Lycopodium clavatum*, *L. annotinum*, und *L. Selago*," which appeared in *Flora* in 1910.

Although botanists, from the time of HOFMEISTER, have tried to germinate the spores of *Lycopodium*, no one but BRUCHMANN ever succeeded with the difficult species which have subterranean prothallia. Some may have failed by throwing away their cultures too early, for the spores of *L. Selago* germinated in 3–5 years; the development of archegonia and antheridia was complete only after 6–8 years; while *L. clavatum* and *L. annotinum* were even slower, germinating after 6–7 years, and requiring 12–15 years to produce an egg ready for fertilization. A long series of cultures in the laboratory, with checks in the field, finally enabled him to give a complete account of the germination of the spore,

development of the gametophyte, fertilization, and embryogeny. BRUCHMANN'S success stimulated others, and while no one else found any prothallia of European species, SPESSARD found American species, HOLLOWAY found prothallia of New Zealand species, while both HOLLOWAY and LAWSON discovered the prothallia of *Tmesipteris* and *Psilotum*.

BRUCHMANN naturally became interested in other subterranean prothallia, and succeeded in finding prothallia of *Ophioglossum vulgatum* and *Botrychium Lunaria*, both of which he described in his usual thorough manner. *Selaginella*, since it is a lycopod, was investigated, although its prothallia are not subterranean. He also made a study of the behavior of the sperms of lycopods with special reference to chemotaxis.

The paper of 1898 brought widespread recognition, for in 1899 he received the Plato Medal of the Academy of Science of Munich, and in the following year the Demazières Prize of the Paris Academy of Sciences, which carries with it a monetary consideration of 1500 francs. Still later he was made an honorary member of the Naturforschende Gesellschaft of Berne. In a letter received by the writer in 1911, however, BRUCHMANN states that, while the prizes are gratifying, his greatest satisfaction is in the recognition his work is receiving in textbooks which bring his results before students in the schools. His experience as a teacher prompted him to prepare splendid sets of prothallia for laboratory demonstration, and these are now used in most German universities and in many universities of other countries.

BRUCHMANN'S life and patient, persistent work prove that one who has the interest and will to do research work can achieve a high rank in science without the stimulus of a great university.—CHARLES J. CHAMBERLAIN, *University of Chicago*.

CURRENT LITERATURE

BOOK REVIEWS

Actinomycetes

The last decade has witnessed the publication of a considerable number of lengthy papers dealing with one or more species of the genus *Actinomyces*. None, however, has equaled, either in volume or in scope, LIESKE'S[1] recent book, in which are incorporated the results of seven years of research, besides considerable information obtained from the very extensive bibliography, of which nearly 400 titles, representing only a part of the total, are cited. The largest section of the volume is devoted to a treatment of the physiological properties of the Actinomycetes, including their reactions to nutrient and tonic compounds, their production of odors and pigments, their enzymatic activities, as well as a discussion of variations of different strains of *Actinomyces* arising in response to changed conditions, or quite spontaneously under uniform conditions. The spontaneous variations with respect to chromogenesis, sporulation, oxygen requirements, thermal relations, and production of odors, the author regards as being in the nature of mutations. The two final sections of the book deal with the relation of the group of organisms to animal and human diseases, and to the diseases of higher plants. In connection with the latter, the galls of alder roots are discussed, and a certain amount of evidence, unfortunately not altogether conclusive, is adduced to show that the causative organism is a species of *Actinomyces*.

In the Preface, the author expresses the justifiable hope that the book may have been made to embrace both the botanical and the medical provinces of bacteriology. He sees in the methods of medical bacteriology a more highly developed technique, from the use of which botanical investigations might profit. Accordingly it is not surprising that the research reported in the book, not excluding the section on morphology, is the product of the established type of medical bacteriological technique, although the latter thus far can hardly be credited with having revealed much concerning the structure of any group of microorganisms. The material to be studied appears largely to have been crushed or smeared on the slide, fixed by heating, and stained by Gram's method. The author concludes that septa are absent from the aerial sporulating filaments, which in view of the fact that the stains used fail to show the walls of fungi, even when these are clearly visible in unstained preparations, need occasion no astonishment. It is to be regretted that some stain known

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[1] LIESKE, RUDOLF, Morphologie und Biologie der Strahlenpilze (Actinomyceten). 8 vo. pp. ix+292. *pls. 4* (colored). *figs. 112*. Leipzig: Gebrüder Borntraeger. 1921.

to affect wall material, as, for example, Delafield's haemotoxylin applied for several hours, was not tried. The process of sporulation is held to be similar to the division of bacteria, and is generally referred to as a breaking up ("Zerfall") of the filaments, both aerial and submerged. It appears difficult to understand why the irregular degenerative structures developed in submerged material, shown in fig. 49, should be designated as spores at all. In fig. 44, showing the development of aerial spores, sporulation is represented as involving the filament below the point of insertion of a branch, a condition which perhaps it might be not at all easy to find realized in any preparation. A new type of spore is also described, the "Vierhyphenspore." The development of the latter is initiated by the proliferation of two short branches at right angles near the tip of a filament. The four elements about the intercalary portion then assume symmetrical positions with reference to it, and yield their contents to the intercalary portion, the latter thus becoming the spore. Although nuclear fusions were not observed, the author believes it probable that some sort of sexuality may be present, the figures observed appearing to show some similarity to the so-called zygospores of certain microorganisms. In spite of the remarks of the author, the figures illustrating these structures do not impress the reader as anything especially distinctive, and he is left to wonder why the author saw here a character recalling the fungi, when he failed to find fungus characteristics in the incomparably more distinctive sporogenous apparatus.

In general the author seems inclined to minimize the significance of such fungus-like characteristics as are revealed even on smear preparations stained according to GRAM. He recognizes in the Actinomycetes a group of organisms occupying an independent position between the fungi and the bacteria, but more closely related to bacteria, particularly to those of the acid-fast type. As to a taxonomy of species, he offers little in the way of encouragement to followers of precedent. The concept of species he holds to be utterly impossible to apply here, all strains showing an exceptional degree of variability under different conditions, and the presence of intergrading strains bridging over whatever differences may be observed between extremes. Moreover, the different strains are said to change their physiological and morphological characteristics in relatively short periods of time, a circumstance that would rob any attempt at classification of any except a slight historical interest. Even the recognition of group species, certainly not a very happy conception at best, is held to be futile for the same reasons. One might desire the author to have extended his observations on the tendency toward mutation, to include besides characteristics like color of thallus, spore color, or abundance of sporulation, in respect to which it is almost impossible to get any group of fungi to behave in a consistent way, possible significant changes in structure. As the direction of relation of the spiral sporogenous hyphae, for example, has been reported to be an invariable specific characteristic, it would have been interesting to learn whether or not this too is subject to change by mutation.

The volume contains an abundance of illustrations, including a large number of microphotographs of excellent quality, as well as four plates of colored figures very well executed and reproduced.—CHARLES DRECHSLER, *New York Botanic Gardens.*

Rocky Mountain flowers

Since the appearance of its earlier edition in 1914, none of the less technical books have proved as useful in becoming acquainted with the vegetation of the Rocky Mountains as Clements' *Rocky Mountain flowers.*[2] This is due in large measure to the drawings, and more especially to the attractive colored plates that are reproductions of water color sketches by Mrs. CLEMENTS. The twenty-five colored plates, together with a very simple descriptive text, have also been issued in a smaller volume[3] for the use of travelers, or to be employed as a souvenir of vacation days.

Among the notable features of the larger volume are a chart exhibiting the genetic relationship of plant families, a key to the families, and numerous keys to genera and species. More complete descriptions of species would be desirable, but could only be given by greatly enlarging the volume. The present edition is on thinner paper, and being bound in flexible leather covers, is very convenient as a pocket companion on mountain climbs.—GEO. D. FULLER.

Weeds of farm land

Miss BRENCHLEY[4] has published an interesting book on weeds of farm land. This discussion of farm weeds should be interesting to the practical man as affording an opportunity of comparison between the problems of the English and the American farmer. For the latter there may be practical hints and methods that may be applicable to his own fields. The ecologist will find more discussion of the fundamental problems of competition and succession than generally appears in weed manuals. The chapters on "Vitality of weed seeds," "Association with soils," "Association with crops," and "Grass land weeds" touch upon the relationship of plants to their environment and to one another, and seek for solutions of practical problems in the control of limiting factors. Other interesting chapters are those on "Parasitic weeds," "Poisonous weeds," "Uses of weeds," and "Popular and local names of weeds." The last topic is treated in detail, not less than 69 synonyms being given for *Galium Aparine*, 80 for *Orchis mascula*, 54 for *Senecio Jacobaea*, 35 for *Polygonum aviculare*, and 40 for *Ranunculus acris*. The illustrations suffer by comparison with those of similar American books.—GEO. D. FULLER.

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[2] CLEMENTS, F. E., and CLEMENTS, EDITH S., Rocky Mountain flowers. 8vo. pp. 392. *pls. 47.* Field edition. New York: H. W. Wilson Co. 1920.

[3] CLEMENTS, EDITH S., Flowers of the mountain and plain. 8vo. pp. 79. *pls. 25.* New York: H. W. Wilson Co. 1920.

[4] BRENCHLEY, WINIFRED E., Weeds of farm land. 12mo. viii+239. *figs. 41.* London: Longmans Green & Co. 1920. $4.50.

NOTES FOR STUDENTS

Taxonomic notes.—BLAKE[5] has described a new genus (*Neomillspaughia*) of Polygonaceae, based on *Campderia paniculata* Donn. Sm., of Honduras. It includes another species from Yucatan.

PENNELL[6] has begun the publication of a list of genera of RAFINESQUE not recorded in *Index Kewensis*, although published in *Autikon Botanikon*. This first instalment presents 83 genera which should be included in any complete index. It is possible that some of these names should be in use.

YUNCKER[7] has published a very complete revision of *Cuscuta*, which is the first attack upon this difficult genus since ENGLEMANN's monograph of 1859. The history and morphology of the genus are given, in addition to the taxonomic presentation. The monograph includes 54 species, 26 occurring in the United States, 33 in Mexico, and 7 in the West Indies. Of the 54 species and 42 varieties, 14 species and 16 varieties are new, and 32 species are figured for the first time. A full bibliography and index of collections are also included.

MISS DOIDGE[8] has published the results of her studies of South African Microthyriaceae, adding very materially to the knowledge of the group in that region. She recognizes 24 genera, including 50 species, 28 of which are described as new. Much the largest genus is *Asterina*, with 30 species, 14 of which are new. The remaining new species are distributed among 9 genera.

STANDLEY[9] has published a manual of the flora of Glacier National Park, especially for the benefit of the numerous visitors. The manual will be of use also elsewhere in the mountains of Idaho, Alberta, and British Columbia, and will be helpful in the Yellowstone National Park.

TRELEASE[10] has monographed the North American species of *Piper* belonging to the section OTTONIA. He recognizes 12 species, describing 8 of them as new.

The second contribution to the flora of Micronesia and Polynesia,[11] under the editorship of DIELS, includes 24 contributions by various investigators. The most extensive one (68 pp.) is by SCHLECHTER on the Orchidaceae

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[5] BLAKE, S. F., *Neomillspaughia*, a new genus of Polygonaceae, with remarks on related genera. Bull. Torr. Bot. Club **48**:77–88. *pl. 1.* 1921.

[6] PENNELL, F. W., "Unrecorded" genera of RAFINESQUE. 1. Autikon Botanikon (1840). Bull. Torr. Bot. Club **48**:89–96. 1921.

[7] YUNCKER, T. G., Revision of the North American and West Indian species of *Cuscuta*. Univ. Ill. Biol. Monographs **6**:1–142. *pls. 13.* 1921.

[8] DOIDGE, ETHEL M., South African Microthyriaceae. Trans. Roy. Soc. S. Africa **8**:235–282. *pls. 13–19.* 1920.

[9] STANDLEY, PAUL C., Flora of Glacier National Park, Montana. Contrib. U.S. Nat. Herb. **22**:235–438. *pls. 33–52.* 1921.

[10] TRELEASE, W., North American Pipers of the section Ottonia. Amer. Jour. Bot. **8**:212–217. *pls. 5–8.* 1921.

[11] Beiträge zur Flora von Mikronesien und Polynesien. II. Engler's Bot. Jahrb. **65**:429–528. 1921.

of Micronesia. He recognizes 37 genera, one of which (*Rhynchophreatia*) is new, and describes 38 new species. We are only beginning to realize the wealth of orchids in the tropics.

HUGHES[12] has published a revision of the Australian species of *Stipa*, recognizing 40 species, 17 of which are described as new. This is in striking contrast with the 15 species recognized in the *Flora Australiensis*, especially since only 5 species of the 40 characterized are based on material unknown to BENTHAM.

The current numbers of *Notizblatt* (Bot. Gart. Berlin-Dahlem) contain numerous taxonomic contributions, dealing with the flora of South America, Africa, and the East Indies. The new genera described are as follows: *Tetradema* (Gesneriaceae) by SCHLECHTER (7:15–18. 1920), from the East Indies and the Philippines; *Peekelia* (Leguminosae) by HARMS (7:26, 27. 1920), from New Guinea; *Chelyocarpus* (Palmaceae) by DAMMER (7:44–51. 1921), from Brazil; *Paraphyadanthe* (Flacourtiaceae) by MILDBRAED (7:399–405. 1921), from Africa; *Cheilanthopsis* (Polypodiaceae) by HIERONYMUS (7:406–409. 1920), from Burma; *Afrolicania* (Rosaceae) by MILDBRAED (7:483–485. 1921), from Africa; *Neozenkerina* (Scrophulariaceae) by MILDBRAED (7:491–493. 1921), from Africa; *Stenodrepanum* (Leguminosae) by HARMS (7:400–501. 1921). KRÄNZLIN (7:412–451. 1920) also describes 44 new species of Orchidaceae from Columbia, this being only the first paper of a series.—J. M. C.

Origin of Hawaiian flora.—Because of its notable endemism, the flora of the Hawaiian Islands has always been of fascinating interest to plant geographers. CAMPBELL[13] in some recent studies of this flora regards the Hawaiian problem as the most important distributional problem that exists anywhere. HILLEBRAND, and perhaps most investigators, have held that the Hawaiian flora has always been isolated, the islands having been thrown up from great depths by volcanic action. Recent studies by PILSBRY on the Hawaiian land snails have shown noteworthy Malaysian affinities, and now CAMPBELL finds similar evidences from the plants. The liverworts and filmy ferns in particular are unsuited to long overseas transportation, and must have existed in Hawaii since it was connected with other lands. The relationship of these plants is much closer to the flora of Malaysia and Australasia than to America. Of 40 species of pteridophytes found elsewhere, 38 are common to Australasia or Malaysia, and only two are common to America. Fifty-one genera of spermatophytes are common to Australasia or Malaysia, and only six are common to America. The endemic genera are more closely related to Asia

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[12] HUGHES, D. K., A revision of the Australian species of *Stipa*. Kew Bull. no. 1. pp. 30. 1921.

[13] CAMPBELL, D. H., The origin of the Hawaiian flora. Mem. Torr. Bot. Club 17:90–96. 1918.

———, The derivation of the flora of Hawaii. Leland Stanford Junior Univ. Publ. I. pp. 34. 1919.

or the south Pacific than to America. The American elements that are present are accounted for partly through introduction by winds or migratory birds, and partly as a residue of once more widespread forms that are now extinct except in Hawaii and America. The absence of conifers may similarly be explained by extinction, if they were ever present, or by the absence of suitable soil conditions. The almost complete absence, for example, of granitic or calcareous soils might well explain certain absences. It is noted also that great ocean deeps separate Hawaii from America, whereas it is much shallower between Hawaii and the Orient. It is concluded, therefore, that the Hawaiian flora has been derived for the most part from the southern Pacific region, and that the Hawaiian Islands are a remnant of a northeastern extension of some large land mass, once connected closely with south Pacific lands.—H. C. COWLES.

Studies of cambium.—BAILEY,[14] in a third paper on cambium, has made what he calls a cytological "reconnaissance." In the preceding paper, reviewed in this journal,[15] he called attention to the size variations of cambial initials, and to the unusual opportunity offered by the cambium for the study of a number of fundamental cytological problems. In this preliminary study he has reached the following conclusions. The initials of the cambium, which may attain a length of more than 9000 μ, are uninucleate, and the "working distance" of their nuclei must extend in some cases for a distance of several thousand microns. The nucleo-cytoplasmic ratio may be relatively constant in ray initials, but varies enormously in fusiform initials. All the cambium initials of *Pinus Strobus* contain the diploid number of chromosomes. Small ray initials may contain as large chromosomes as adjacent fusiform initials with a volume 200–1000 times as large. Fusiform initials, which are frequently several hundred times as long as they are wide, divide longitudinally by an extraordinary extension of the cell plate. The various types of cell plate formation described by various cytologists are believed to be merely different phases or stages of a single general type of cytokinesis. These glimpses would seem to justify the writer in his belief that the cambium well deserves intensive cytological investigation.—J. M. C.

Economic plants of Philippines.—In an illustrated report BROWN[16] gives a series of descriptions of the indigenous food-producing plants of the Philippines. Many will be surprised to find the statement that the edible wild plants of these islands are less abundant, more inaccessible, and inferior in

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[14] BAILEY, I. W., The cambium and its derivative tissues. III. A reconnaissance of cytological phenomena in the cambium. Amer. Jour. Bot. 7:417–434. *pls. 26–29*. 1920.

[15] BOT. GAZ. 71:408. 1921.

[16] BROWN, WM. H., Wild food plants of the Philippines. Phil. Dept. Agric. and Nat. Res., Bur. For. Bull. 21:1–165. *figs. 81*. 1920.

quality to those found in the United States. There are certain notable exceptions, however, as the pili nut (*Canarium luzonicum*), which is abundant and superior to the almond in quality, and the wild mango (*Mangifera caesia*), with its delicious flavor. Nuts, seeds, fleshy fruits, buds, leaves, roots, and tubers are included in the list, and the drawings and photographs used to represent them are of excellent quality.

A companion report by WEST and BROWN[17] deals with native resins and oil-producing plants, which are rather numerous. One difficulty in the utilization of the resins of many of the trees is to be found in the large number of species found in any particular area, making the number of individuals of any one species in any locality rather small. Several of the oil-producing plants give promise of good results under cultivation. In this report, also, the illustrations and descriptions give much botanical information.—GEO. D. FULLER.

Ecological research.—In his report of the work of the Carnegie Institution for 1920, Director MACDOUGAL[18] indicates the lines of research being followed by various members of his staff, and affords hints of results being attained. He has been measuring in detail the growth of certain trees and the accumulation of food material by the potato. CANNON and FREE have continued their investigations of the growth of roots as related to aeration. The factors affecting plants in new habitats have been under investigation by MACDOUGAL, and his results seem to show that species may be the more readily transferred from cool regions to warm, from montane regions to maritime, and from regions of climatic extremes to those of equable climates than the reverse. SHREVE reports progress in a soil temperature survey of the United States and Canada, in his investigations of the arid Avea Valley, and in his explorations of the Santa Lucia Mountains. Mrs. SHREVE has studied seasonal changes in the transpiration of *Encelia farinosa*, and VINSON and GRIFFIN have investigated the changing composition of Salton Sea water. The strand vegetation near Monterey, California, has been examined by COOPER, and stations and quadrats established for more exact studies of the associations and their controlling factors. Evaporation rates on the Monterey peninsula are decidedly less than in the oak and chaparral region east of Monterey, and this may account for the pine forests covering the former area.—GEO. D. FULLER.

Calcicoles.—In a discussion of plants found on soils supposed to be calcareous, SALISBURY[19] makes it clear that the problem of the limitation of the

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[17] WEST, A. P., and BROWN, W. H., Philippine resins, gums, seed oils, and essential oils. Phil. Dept. Agric. and Nat. Res., Bur. For. Bull. **20**: 1–230. *figs.* 73. 1920.

[18] MACDOUGAL, D. T., Department of botanical research. Carn. Inst. Wash. Year Book for 1920. **19**: 49–81. 1921.

[19] SALISBURY, E. J., The significance of the calcicolous habit. Jour. Ecol. **8**: 202–215. 1920.

species to this substratum is by no means a simple one. In the first place, there is great need of more accurate data regarding the exact distribution of such "calcicoles" and of the exact nature and chemical reaction of the soils in which they are growing. As an example of the need of such precautions it is shown that "calcifuges" may and do occur on soils usually considered calcareous, but on account of leaching there is really no calcium in the soil in contact with the plant during its youthful and critical stages. It is further shown that complexity is added to the problem by the secondary characters usually accompanying calcareous soils, such as their comparative freedom from toxic products of decay, their usually low water-holding capacity, the more abundant development of their soil fauna, and the influence of calcium upon the absorption of other elements such as potassium.

The entire discussion is a thoughtful consideration of the various aspects of the problems concerned, and with the rather extensive bibliography is a good survey of the entire field.—GEO. D. FULLER.

Forest trees of Hokkaido, Japan.—Recognizing in the rapid changes taking place in Hokkaido a menace to the existence of its forests and its timber supplies, the government has appreciated the importance of a scientific knowledge of its trees as a basis for increased attention to forestry. As a result of the investigations thus prompted, there is being issued a most attractive set of beautifully colored plates, accompanied by descriptive text in Japanese and English.[20] The plates depict the foliage, flowers, fruit, buds, seeds, and seedling stages, one plate being devoted to each species. The three fascicles now issued include *Taxus cuspidata*, *Abies sachalinensis*, *A. Mayriana*, *A. Wilsonii*, *Picea Glehni*, *P. jezoensis*, *Larix dahurica*, *Pinus pentaphylla*, *P. pumila*, and *Thujopsis dolabrata*. The finished work will comprise not less than 85 species.—GEO. D. FULLER.

Notes on Conifers.—Two botanical memoirs by CHURCH[21] will be of interest to teachers of botany, especially those most concerned with morphology and forestry. These papers are used at Oxford in class work, making it unnecessary for the students to take lecture notes, and, at the same time, furnishing very complete outlines for laboratory work. Both papers lay emphasis upon features which can be seen without a compound microscope, although the microscope is used for some details of the life history. The first paper is

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[20] MIYABE, KINGO, and KUDO, YUSHUN, Icones of the essential forest trees of Hokkaido. 10.5×15 inches. Sapporo. Pub. by the Hokkaido government. Fasc. 1.1–15. *pls 1–4*. 1920; Fasc. 2.16–26. *pls. 5–7*. 1920; Fasc. 3.27–37. *pls. 8–10* 1921.

[21] CHURCH, A. H., Elementary notes on Conifers. Botanical Memoirs. no. 8. Oxford University Press. pp. 32. 1920.

———, Form factors in Coniferae. Botanical Memoirs. no. 9. Oxford University Press. pp. 28. 1920.

more elementary and would be used by students who have had only a general course in botany. The second paper is more advanced and could be appreciated only by students who have some previous knowledge of Gymnosperms.—C. J. CHAMBERLAIN.

Indian Botanical Society.—A notable botanical movement in India is the recent organization of "The Indian Botanical Society," whose aims, constitution, and list of members have just been published for distribution. It is stated briefly to be "a society for uniting the botanists and promoting the botanical interests of India." A more detailed statement of aims is to improve the quality and content of botanical instruction, to encourage and promote research, to provide a central exchange, and to make available to members the scattered and insufficient botanical literature that reaches India. The president is WINFIELD DUDGEON of Ewing Christian College, Allahabad City, and the other officers, three of whom are Indians, represent other institutions. The society begins with 85 members, representing 10 provinces of India.—J. M. C.

African veld.—In a description of the vegetation of South Africa, POLE-EVANS[22] uses the term "veld" to include all the native vegetation ranging from a rich forest on the southeastern coast to a desert in the interior Karroo. He covers the ground as in a former article noted in this journal,[23] but with more emphasis on the economic resources and possibilities of each region. The nineteen divisions into which he divides the region possess rainfalls ranging from zero to 70 inches per annum, while the diversity in vegetation is correspondingly great. This diversity is made evident by excellent illustrations, as well as by lists of species and the enumeration of resources of timber, fibers, gums, and fruits in addition to the forage plants.—GEO. D. FULLER.

Embryogeny.—SOUÈGES,[24] in continuation of his numerous detailed studies of the embryogeny of various families of seed plants, has reported his results for *Urtica pilulifera*, *Senecio vulgaris*, four species of *Rumex*, and a species of *Rheum*. The details are too numerous to recite, but the excellent figures present the facts clearly for those using such data.—J. M. C.

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[22] POLE-EVANS, I. B., The veld: its resources and dangers. So. African Jour. Sci. **17**:1–34. *figs. 56*. 1920.

[23] BOT. GAZ. **66**:539. 1918.

[24] SOUÈGES, RENE M., Embryogénie des Urticacées. Dévelopement de l'embryon chez *l'Urtica pilulifera*. Compt. Rend. **171**: no. 21. 1920.

———, Embryogénie des Composées. Les premiers stades du développement de l'embryon chez le *Senecio vulgaris*. Compt. Rend. **171**:254. 1920.

———, Embryogénie des Composées. Les dernier stades du développement de l'embryon chez le *Senecio vulgaris*. Compt. Rend. **171**:1920.

———, Recherches sur l'embryogénie des Polygonacées. Bull. Soc. Bot. France IV. **20**:1–11, 75–85. 1920.

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PEAT DEPOSITS AND THEIR EVIDENCE OF CLIMATIC CHANGES

ALFRED P. DACHNOWSKI

(WITH TWELVE FIGURES)

The time in which the various peat deposits of the United States were formed can be determined only from a joint consideration of glacial geology, climate, and plant remains. These reflect the relations between a deposit of peat materials and its environment. To attempt a correlation of this kind on a chronological basis, however, has many difficulties, which investigators in the respective sciences appreciate.

The essential nature of stratigraphic differences in peat deposits is indicated by the nature of the plant remains and the order in which layers of peat material lie upon one another, that is, by the sequence of the vegetation units which at one time formed layers of plant remains in the deposit. As to the tectonic order of the layers or series of layers of material composing a peat deposit, little need be said at this time. From the standpoint of stratigraphy the condition of the initial area in which a pioneer plant population established itself is the critical factor of greatest importance, so far as the beginning of the course of development is concerned. The sequence of the development may become changed anywhere in the course, either by changes in environmental factors or in plant population. These changes are all recorded within the deposit. In the vast majority of peat deposits the beginning of

development and the succession of peat materials have to do with the factor of water content in the original area. Usually the quantity of water is more frequently concerned than its salinity, acidity, or alkalinity. The initial water relation, by its selective action, determines not only the characters of the life forms which establish themselves as the pioneer population, but also the number of layers possible and the order of their sequence.

In a preceding paper (**7**) it was proposed to classify peat deposits of whatever nature into two great primary groups, the group of water-laid peat deposits and the group of land-laid peat deposits, in accordance as they have arisen in water or on partly drained but relatively moist initial areas. In the water-laid peat deposits the bottom layers consist of materials which accumulate only in standing water. They contain the remains of planktonic organisms and macerated material from plants more or less submerged or floating, or which occupied the margins of the basin. Subdivisions of this group are given in the section which follows. In the land-laid group of peat deposits the origin is indicated in the mineral substratum by the vertical roots of plants which at one time occupied the area, as a well defined plant population or vegetation unit. The general stratigraphic subdivisions in this group are indicated by the order in which the vegetation units invaded and occupied the land area. With respect to the layers of peat material formed by them the order may be (1) progressive, that is, beginning with some member of the marsh group of peat materials until the deciduous or coniferous forest climax of the region is reached; (2) stabilized, that is, it may begin and continue in a stable forest climax; or (3) the order may indicate the conversion of the basal forest climax into marsh and finally to open water conditions by the influence of various environmental causes. This distinction between the two primary groups of peat deposits is clear cut, and is readily made in field work. The only possible difficulty arises when the plant remains have been redeposited or partially removed by any later action, such as erosion. Even secondary disturbances of this nature, however, do not invalidate the importance of the stratigraphic viewpoint. Its significance for correlation studies has been sufficiently dwelt upon elsewhere (**6**).

The following peat deposits are representative of the subdivisions in the land-laid group, and will be reported in another paper: 1. The New Haven Marsh near Plymouth, Ohio (glacial Lake Maumee type); the peat deposit southwest of Rome, New York (glacial Lake Iroquois type); and the Algoma Muskeag near Roseau, Minnesota (glacial Lake Agassiz type). 2. The Dismal Swamp west of Norfolk, Virginia (Pamlico coastal terrace type). 3. The Kankakee Marsh, between South Bend and Crumstown, Indiana (in the Bloomington morainic system). It will be noted that peat deposits are regarded here in their relative space and time dimensions.

In regard to the stratigraphic units of peat deposits, reference may be made to Bulletin 802 (5) and left with this passing suggestion. Whatever system of classification of peat materials may be adopted, it will be found that for several reasons it cannot be carried uniformly and with constant value over so broad a territory as discussed here. The main difficulty arises from the unlike development of the vegetation unit which forms the layer of peat, and from modifications of the successional series in diverse geographic regions. Insensible gradations or phases due to variations in composition of plant remains set a limit to the most refined botanical division of peat materials that can be recognized.

Investigators approaching peat-land problems for the first time are apt to be influenced by the idea of permanence and fixity of specific limits. In a large measure this may be accounted for by the fact that the very recognition of such a thing as a type of peat material carries with it the impression of an entity, and that, if these characteristics are modified or supplanted by others, the unit in question no longer belongs to that type. The degree of individual difference admissible within a type is a matter of individual judgment. Variations exist within specific limits, but what these limits are is still a matter of diverse and constantly changing opinion, until these gradations and phases are measurably well established. No evidence of this sort of peat type limitation is available as yet, but the detailed application of ecological and instrumental methods strengthens the conviction that the

arrangement and naming of the different types of peat are merely matters of practice in field work.

In this paper it is not the intention to furnish the numerous details necessary to a knowledge of the different types of peat material, nor is it necessary to review from a voluminous European literature all the widely scattered observations on types of peat and their variations. So far as observations indicate, variations of stratigraphic units represent but a temporary condition. The structural development of a peat deposit is characterized by the regular occurrence of several types of peat material in many different forms and phases, such as differences in the growth and evolution of vegetation units. These phases are connected by more or less constant field relations. Unquestionably many so-called ecological stages represent merely fragments in the development of a peat deposit, reactions of one plant population upon another. On the other hand, well distinguished types of peat material will not only keep their position, but will receive a much more nearly complete and sharper definition than they have at present. It is for these reasons that only major divisions of plant remains are distinguished in the following discussion. They have been adopted also wherever the differences of information are sufficient to occasion difficulty in applying a uniform classification of types of peat. The list has been summarized (**5**), and has been utilized with the addition of two new marsh types of peat found in Florida and California respectively, to facilitate reference between the cross-sections of peat deposits and the text.

The conventional signs represented in the graphic illustrations of the profile sections described in large part are adjusted to the standard of European workers and the requirements of cartography. The departures which arise (partially from the inaptness of the material as a type of peat) have been stated in the legend, and in connection with each layer described in the text. Beds or strata which are not sharply defined in a deposit may be recognized by the dotted boundary lines.

It is to the interest of a group of scientific and industrial workers that coordinated efforts should be brought to the solution of peat-land problems. To those who desire general field information

regarding types of plant remains the following are some of the localities near which layers of peat material are displayed in typical form at or somewhat below the surface of the peat deposit. Macerated and colloidal types in Cedar Lake near Fremont, Indiana (fig. 2); *Phragmites* type and *Carex* type near reservoir on New Haven Marsh, Plymouth, Ohio; *Hypnum* type in Algoma Muskeag near Roseau, Minnesota, and in basal layer of the peat deposit exposed along the barge canal and James Street bridge below Rome, New York; *Cladium* type in the Florida Everglades at Okelanta and vicinity; *Scirpus* type at Middle River and near Wintersburg, California; *Sphagnum* type on Cranberry Island at Buckeye Lake, Ohio (fig. 3), and in peat deposits west of Arlberg, Minnesota; coniferous forest types near Kent, Ohio (fig. 10), and north of Kelliher and Warroad, Minnesota; mixed deciduous forest litter type in Dismal Swamp, Virginia, in basal forest of Kankakee Marsh near Crumstown and South Bend, Indiana, and in middle and upper forest beds of the peat deposit southwest of Rome, New York; deciduous forest type near Mantua, Ohio (fig. 12). More specific information concerning peat materials and their agricultural and industrial value may be obtained in Bulletin 802 (5).

I. Water-laid peat deposits.

The chief feature of the group of water-laid peat deposits is the presence of aquatic types of peat material as the initial layer. The deposits may vary widely in the number and character of the initial stages, and the number of stages may range from one layer to several in the deeper deposits, including secondary phases. From the manner in which the peat materials are laid down in standing or in flowing water, in fresh or in brackish and saline water, the successive layers as a rule furnish conclusive evidence of three major series of stratigraphic differences. The group of water-laid peat deposits may be subdivided into (1) basin deposits with standing water level, such as lake and pond deposits, and (2) deposits in depressions with fluctuating water level, the river and overflow deposits, of which the Florida Everglades and their alternation of fibrous and macerated layers of peat material are

a notable example. The coastal river and estuarine peat-lands merge into the (3) marine deposits such as tidal marsh and mangrove swamps. A discussion of the brackish and salt water deposits is reserved for a future paper.

EVIDENCE OF CLIMATIC CHANGES

It might seem that the water-laid group of peat deposits could not offer reliable and direct criteria for evaluating age or time correlations, since water in basins constitutes a fairly uniform environment. There is continuity in the sequence of strata of plant remains, but macerated and more or less structureless layers of peat material bear no fixed relation to the plant populations which succeeded each other in the development of the deposit. The organic fragments are derived from many sources, and are in large part from suspended débris. Nevertheless, inferential evidence of past vegetation units and climatic changes may occur in abundance.

The evidence for age and for climatic correlations is of several kinds, of which one form is represented in the scattering and mixing of leaves, pollen, and seeds blown into a peat deposit or washed in from adjacent land vegetation units. The latest substantial comparison between plant remains (such as the pollen of conifers) in layers of peat material and the changes in climate and in the composition of land-plant communities is the quantitative method employed by VON POST (**24**).

A second kind of evidence of climatic changes found in peat deposits consists of dark colored, partly macerated, and fibrous layers of material alternating with predominantly finely fibrous, coarsely fibrous, or woody plant remains. The close association of this kind of stratification in practically all sorts of peat deposits, without any close relation to topography or the influence of animal agencies, appears to signify alternating wet and dry environmental conditions. Quite frequently the dark colored, partly fibrous layer of peat material is referred to by American writers as "well decomposed peat." Although it bears a striking superficial resemblance to a finer texture, similar to weathered surface material, a layer of this character does not imply conditions of aeration, or of warmth

and dryness by means of which decomposition and oxidation are accomplished. The layer represents rather the open scattered growth of plant populations, such as sedges, reeds, rushes, and brown mosses. The "well decomposed" débris as a rule is the intermixture of macerated material from aquatic and amphibious plants. The presence of diatoms, sponge spicules, shells, silt, and windblown material of various kinds usually shows that the chief condition for its formation is a higher water level. So long as the water table continues at a higher level, the fibrous type of peat tends to retain the aquatic admixture; the disappearance of the macerated débris would indicate conditions of ground water below the surface soil; and an alternating sequence of these layers would mark a period of climatic pulsation, of alternating wet and dry conditions. In carrying out quantitative determinations on samples of "well or partly decomposed" peat materials, the use of the colloidal suspension test and the methods of KÖNIG (**15**), MELIN and ODÉN (**22**), and KEPPELER (**13**) are only partly adequate. The possibility of obtaining erroneous results must be checked by a preliminary microscopic examination of the organic material, and by a consideration of its position in the profile structure of the deposit.

Some European workers are strongly of the opinion that a climatic break in the waning portion of the glacial period is indicated by the remains of forests found buried in stratified peat deposits, and by the "horizon" layer between the lower, in part disintegrated, and the upper, relatively more recent sphagnum peat of certain high moors. The materials are believed to be evidence showing there has not been merely a steady amelioration of climate since the last ice movement, but rather a fluctuation between periods of dry and wet climatic conditions. The dissent from this interpretation on the part of other investigators does not appear to be chiefly a matter of the proper terms to apply to types of peat and their variations. These layers of "horizon" peat and of buried forest, however, constitute more properly supra-aquatic types of plant remains, and on that account their consideration is deferred to the section dealing with the general stratigraphic features of lacustrine deposits of peat. In this connection it is suggested that

superimposed layers of colloidal type of peat material in certain deposits probably indicate another kind of evidence of former relatively dry and warm climatic conditions.

A third form of evidence which may aid in the interpretation of the age of deposits and the climate which characterized their development consists in the seams of clay found between layers of peat material. These are often found with an admixture of organic matter, but rarely laminated in a manner similar to the seasonally laminated glacial clays described by DE GEER (**10**) and SAURAMO (**32**). Interstitial clay seams appear to be coincident with the earlier portion of the Wisconsin group of moraines. They have been noted especially in connection with peat deposits located in areas where readvances of the ice sheet are displayed in the drift. The investigations, however, are not sufficiently extensive to show whether the clay seams would be prominent also in morainal systems which are free from a surface cover of wind-blown loess.

The fourth form of evidence which seems very promising is that of the marked structural differences found in certain peat deposits over a wide extent of country in which a series of moraine systems is the time factor of distinction (fig. 1). LEVERETT (**19**) has shown that the Wisconsin drift displays moraines which are distinctive and well preserved. They are more or less concentrated in groups which permit of much greater detail of correlation than is possible in connection with the glacial stages in Europe (**12, 18, 27**).

The morainic systems of the Wisconsin ice sheet mark halting places in the recession of the ice front. They obviously represent climatic pulsations, for the evidence seems clear that the ice sheet was subject to increase or decrease in response to climatic variations. Periods of warmth during which the ice sheet retreated somewhat rapidly, leaving nearly level tracts of drift, must have alternated with periods in which the climate ceased to be mild, and either remained nearly uniformly colder for a time or else reverted toward the conditions which induce glaciation.

For the study of past climatic changes and plant migration since the culmination of the last stage of glaciation, a comparison of the stratigraphic features of peat deposits should bring out evidence of great value. By actual test borings of peat deposits

within the area of the several great morainic systems, such as the Shelbyville, the Bloomington, the Valparaiso-Kalamazoo-Missis-sinawa, the Lake Border–Defiance, and the Port Huron, it should

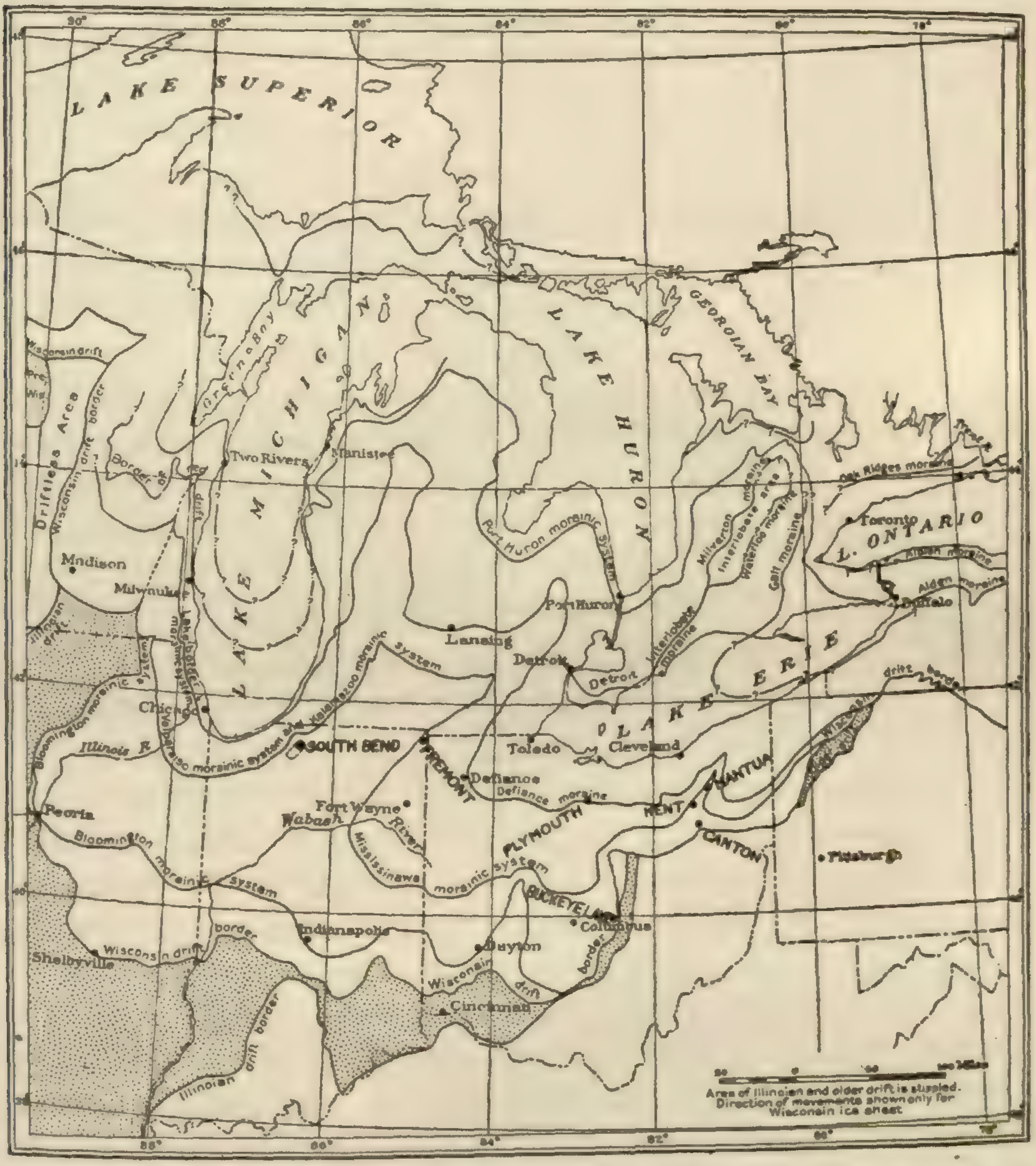

FIG. 1.—Diagrammatic outline of Wisconsin ice border at several successive positions (after LEVERETT and TAYLOR **19** with slight modification): lines of direction of ice movement omitted from original map, and names of a few localities with peat deposits added by writer.

be possible to sum up the whole series of climatic changes which have taken place while the ice field receded, and to estimate the length of time for every single glacial substage. Primarily because

of their greater age, the deposits of the earlier morainic fields constitute climatic indicators of the greatest interest, and they should not only furnish additional data, but also serve as a check upon any evidence which the peat deposits in the later morainic areas may contribute.

The material here presented is only of preliminary import. It has emerged in the field work of the past seven years, and hence a definite correlation is impossible as yet, partly because too little is known of the extent and intensity of the changes. The chief difficulty, however, lies in the fact that much along the line of detailed field and laboratory studies has still to be accumulated. The conclusion is irresistible, however, that when the field is traversed the peat deposits will be found to furnish a new great record of the vegetational and climatic history of the country since Pleistocene times.

General stratigraphic features in water-laid peat deposits

The question of the formation of lacustrine peat deposits has produced a copious literature in many countries, but there is still a dearth of observational evidence on their actual structural origin. Hardly a case exists of an intensive study in which conclusive proof is available showing the types of peat material in process of formation. This does not mean that the process may not be as is generally assumed, but it does indicate that even a well-nigh universal opinion may yet constitute merely an excellent working hypothesis. It can be accepted definitely only after more rigorous tests and extensive field work disclose a clearly defined basis. This account merely serves to emphasize what may be regarded as a general view of the development and structure of lacustrine peat deposits. Although this has been discussed at some length in various papers already published, a brief outline is presented here in order to connect it with the profile sections of the peat deposits on which these discussions have a bearing. The cross-sections which follow have been selected from American and European peat deposits largely on account of their stratigraphic relationship. They visualize the succession of strata in water-laid deposits, and illus-

trate the general development which had come to the final stage possible under the limits of the particular field conditions of different countries.

In a consideration of basined deposits or moors it should be kept in mind that depressions with standing water originate in a great variety of ways (30). Of chief importance, however, is the fact that the initial types of peat material are primarily water-laid. They are largely confined to the lower or deeper parts of the depression, where planktonic organisms, together with comminuted fragments and other plant remains from both land and aquatic vegetation, sink to the bottom. A complete filling of lake and pond basins does not usually occur by the formation of aquatic types of peat material. The peat-land near Fremont, Indiana (fig. 2), represents a relatively rare deposit of peat. The level where the higher plant communities can gain a foothold or succeed one another depends upon the ability of the plants to form a

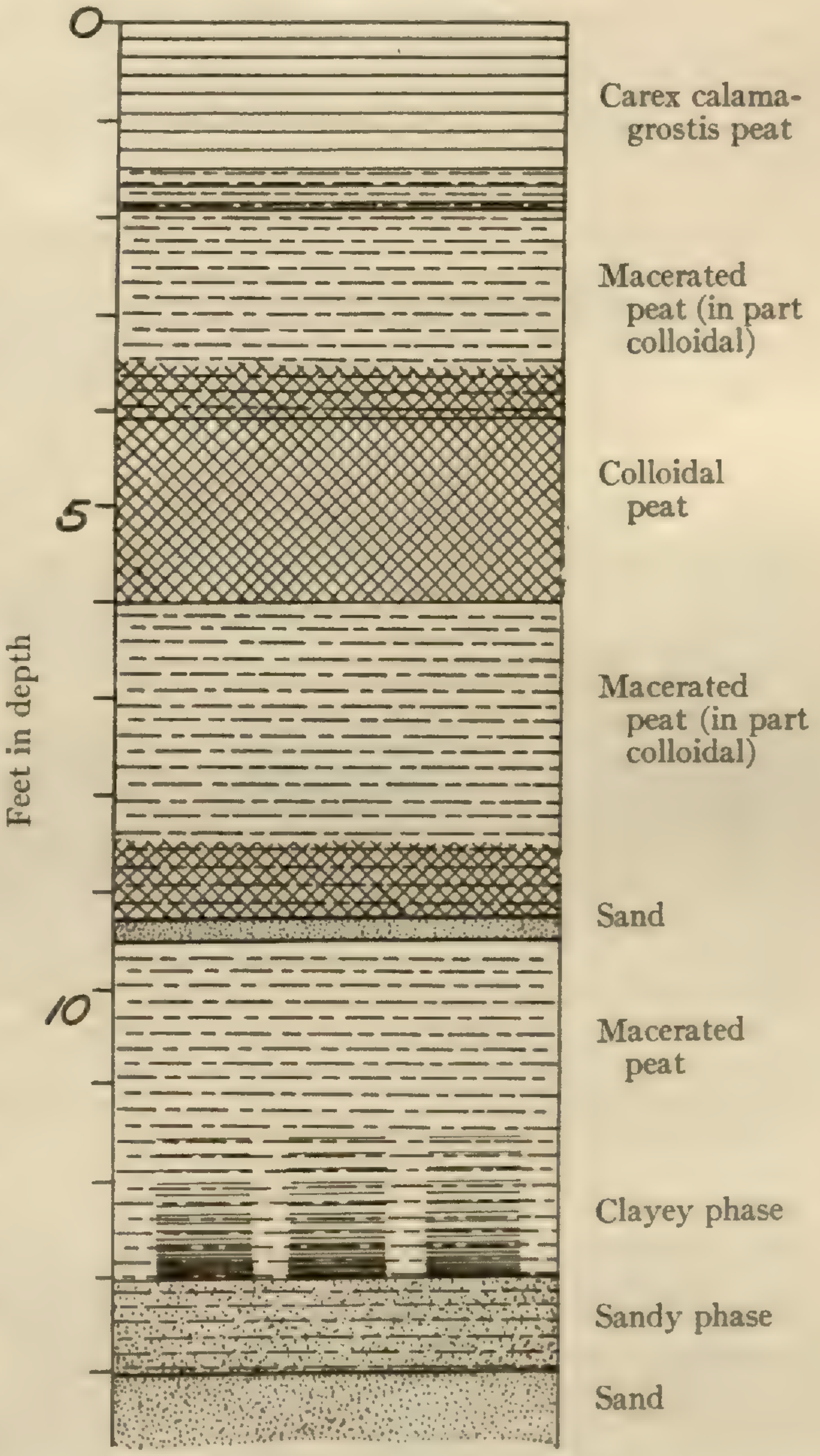

FIG. 2.—Cross-section of soundings in "Cedar Lake" peat deposit near Fremont, Steuben County, Indiana.

floating mat. The thickness of purely allochthonous (transported to the place of occurrence) layers of peat, therefore, is far less extensive than might be assumed, partly also because submerged and amphibious plant populations can take root in depths varying from 10 to 15 feet (3–4.5 m.) and accumulate as peat in situ. The macerated type of peat is nevertheless preeminent, and it varies least in character under conditions which give rise to water colored brown from the presence of suspended and dissolved organic débris. On account of the decrease in light and heat available, and the consequent absence of submersed plant communities, the filling of the depression is chiefly from vegetation units bordering the basin. The colloidal and doppleritic types of peat, on the other hand, make clear another set of conditions; they appear to indicate a higher calcium carbonate content of the waters at the time of their formation, and stimulating environmental conditions of temperature and light, in which the growth of aquatic vegetation units and planktonic organisms probably reached unprecedented proportions. There are reasons for concluding that the colloidal and doppleritic types of peat may represent another kind of evidence of climatic fluctuations. In the deposit near Fremont, Indiana (fig. 2), for example, colloidal material alternates with layers of macerated and "acidic" plant remains. The formation of colloidal material, therefore, may correspond in time with conditions of drought, when the lake or pond waters were concentrated by evaporation and became alkaline as concentration progressed. It is quite probable that the finer calcareous material in the drift had been removed by leaching, and produced variations in the chemical composition of the lake and ground waters. The calcium carbonate content when separating in the open water in a finely divided state must have become mingled with the plant débris so as to form a flocculation product and in places an end product of plant disintegration combined with lime. The climatic changes which brought about this condition may not have been sudden or excessive, but probably were oscillations of moderate intensity, whose cumulative effects were felt during that period of time.

The rate of building up a peat deposit in lakes or ponds appears to increase considerably as a plant population such as that formed by sedges pushes out from the shores, becomes nearly or quite

closed and exclusive, and forms a floating mat. Essentially this mat is fibrous and contains macerated débris. When only partially attached at the sides or beneath the surface, and if for any cause there is a considerable rise of the water surface, the mat floats upon a pocket of water (fig. 3). Later the mat is compact enough to bear the weight of shrubs, trees, and even of dense forests. When, however, the weight of the floating mat becomes too great, it either breaks or sinks with its load. Layers of marsh, shrub, or forest types of peat material then occur, interpolated between layers of aquatic plant remains. Thus an inverted order of superposition results. It would obviously be a fallacy to correlate stratification of this kind with alternating dry and wet climatic periods. Neither would the profile indicate conversion such as may result from artificial causes which obstruct drainage, nor a backward sequence of plant communities, that is, retrogression.

It is apparent also that in the gradual closing of basined

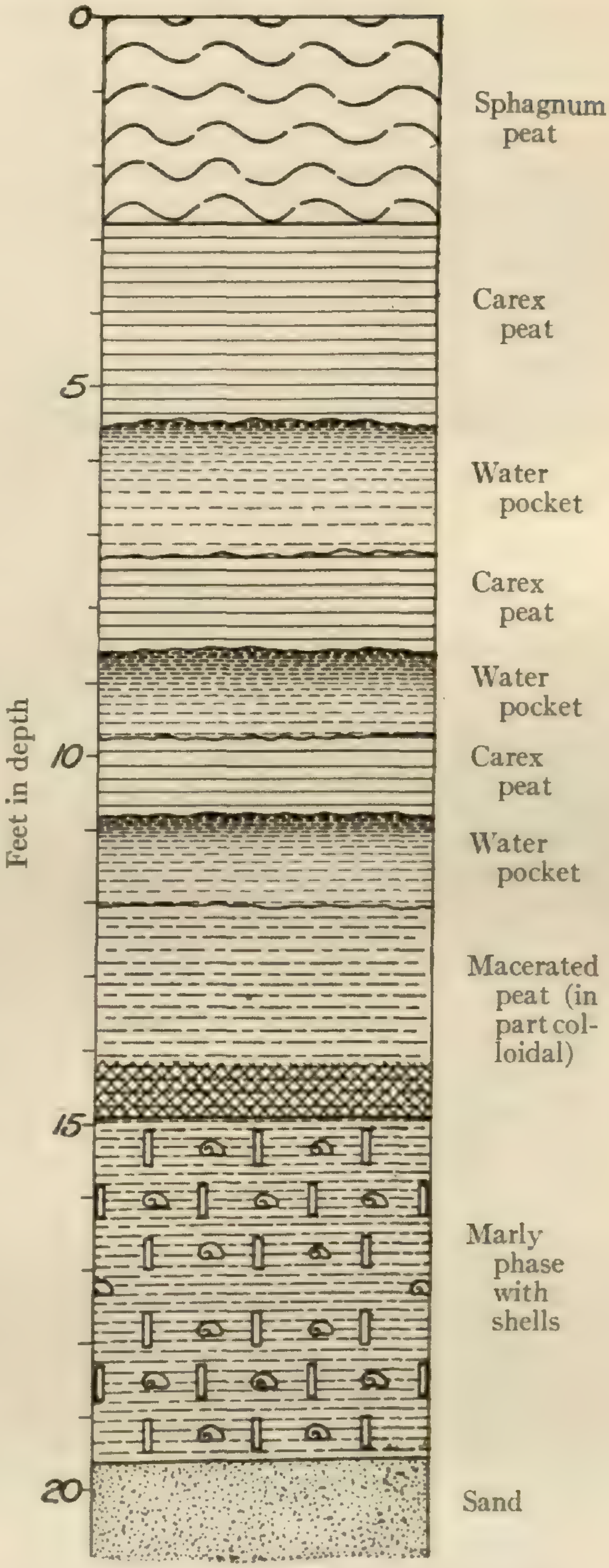

FIG. 3.—Profile section of "Cranberry Island" peat deposit at Buckeye Lake, Licking County, Ohio: elevation 892 feet a.t.; location of sounding near former experiment station (see fig. 4, BOT. GAZ. **52**: 25. 1911); a dike was built in 1838 which raised the water level 8 feet.

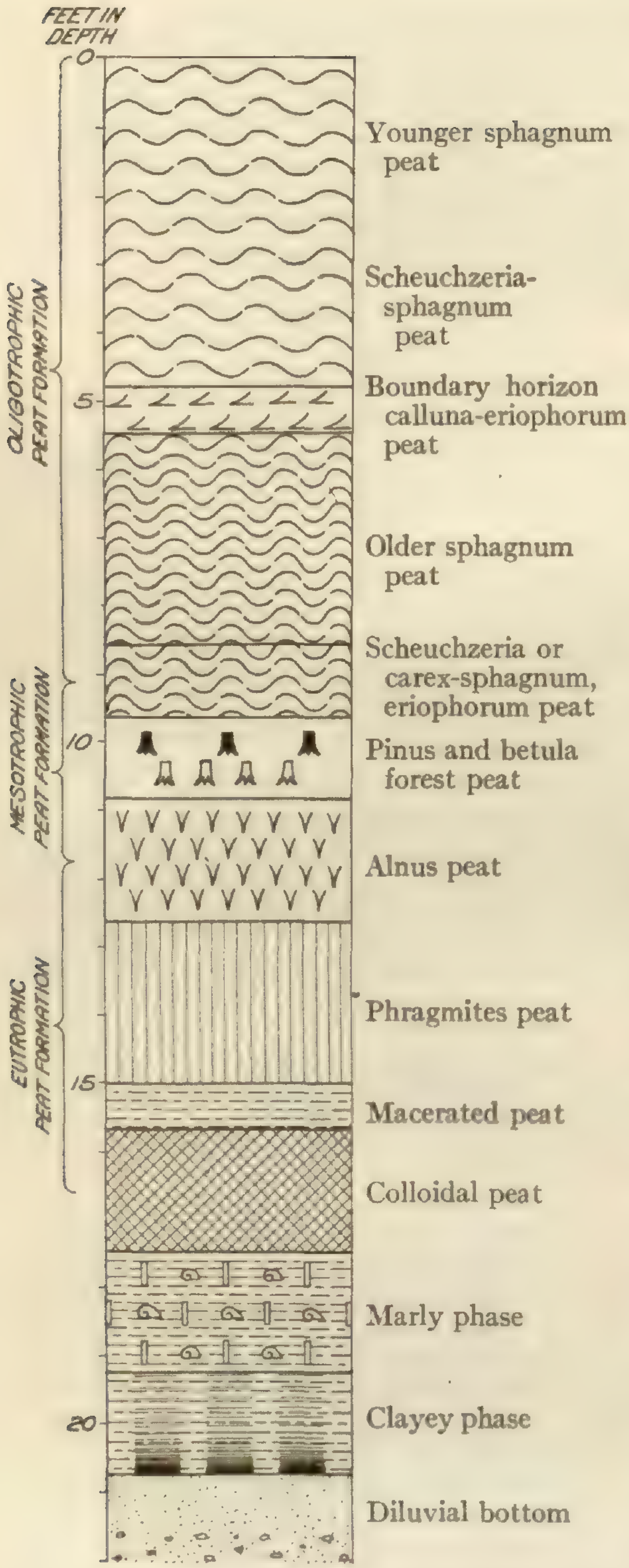

FIG. 4.—Generalized section through North German peat deposit 7 m. (22 feet) in thickness, showing succession of layers of peat material; after WEBER (36).

water by vegetation units there is not only a gradual decrease in available ground water, but in mineral food constituents as well. The earlier plant communities and those which occupy a position near the margin of the basin derive their salts from the water or from the soil on which they grow. For the succeeding units this becomes less and less in amount. When completely filled, the inward sequence of peat material (the horizontal section) and the upward sequence or vertical section of plant remains may show characteristics successively distinct in content of mineral matter, such as lime, and of water incident to the increase of thickness of peat. The difference between the total mineral content of a peat deposit and an adjoining lake has been shown for Cranberry Island at Buckeye Lake, Ohio (3). The term eutrophic is used by WEBER (36) for types of peat formed in water rich in mineral nutrients, and oligotrophic

for types with water poor in saline food constituents, while mesotrophic is applied to the peat materials in the intermediate stage (fig. 4).

Another significant difference lies in the fact that the final climatic vegetation unit of a particular region, for example, a deciduous forest (fig. 12) or a coniferous forest, is also the climax stage of the sequence of peat materials in lacustrine deposits. Successionally the sphagnum and heath shrub vegetation units appear to be a later stage in the structural development of peat deposits. Their superposition upon marsh or forest types of plant remains, however, is not to be considered an anomaly or an exception. The sequence stands in the same causal relation to development as is the case with other vegetation units. Here, however, it is connected with the fact that the ground waters of peat deposits in this stage of development are deficient in mineral salts, and that bog mosses absorb and retain large quantities of rain water on account of their anatomical structure. Sphagnum peat materials reach their greatest thickness in cool humid locations with abundant rainfall, and contain as a rule only little mineral matter. Theoretically the sphagnum stage in the structural development of a peat deposit should be succeeded by shrub, and finally by forest stages in the course of time. Actually this does not appear to take place, unless the layer of moss peat has been reduced in volume by disintegrating processes, and as a result becomes more permeable to ground waters. In the present state of our knowledge it is impossible to be certain that disintegration can occur without a change in climate. Thus the "horizon peat" between the lower (older) and the relatively more recent (upper) sphagnum peat in northern Germany (fig. 4) is regarded by WEBER (**36**) as due to a climatic change unfavorable for the growth of sphagnum mosses. VON POST (**23**) has corroborated this view by his work in Sweden, VAN BAREN (**1**) confirmed it for some of the peat deposits of the Netherlands, and ZAILER (**37**) verified it for the peat deposits of the Enns Valley in the Austrian Alps. The layer is assumed to indicate a long interruption of peat formation, during which the high moor was covered with *Eriophorum* and *Calluna*, and sometimes with forest. WEBER and VON POST conclude that the horizon peat

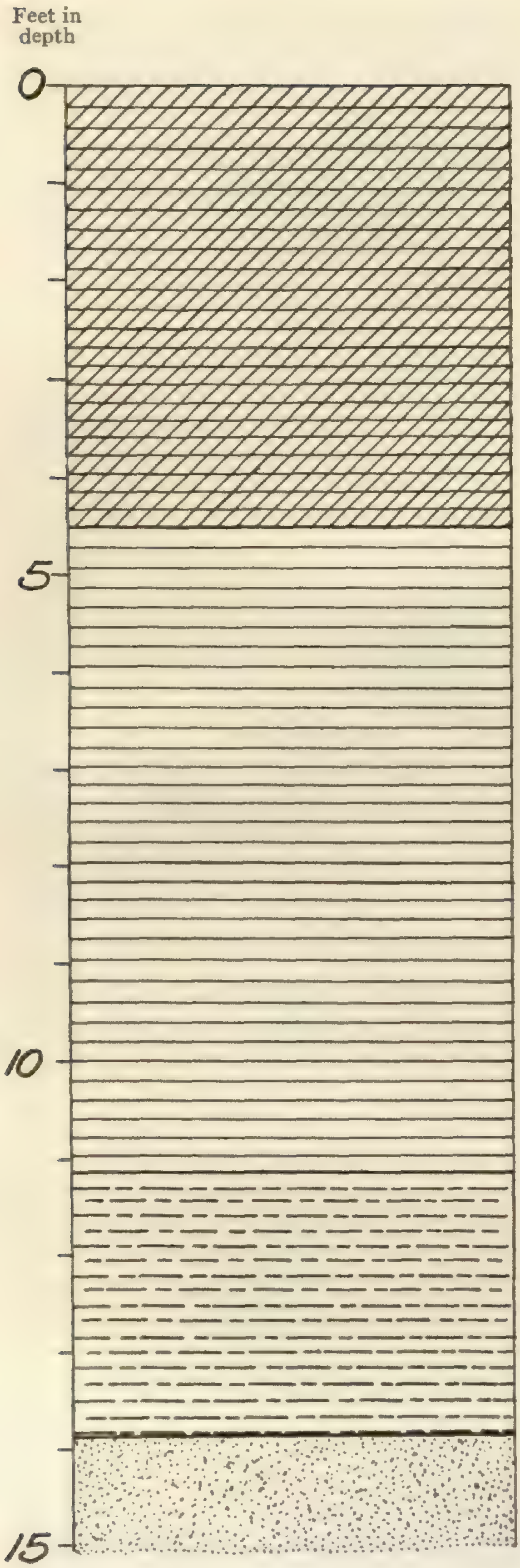

FIG. 5.—Cross-section of peat deposit near Sarna Station, Volinsk Province, Russia; after DOCTUROWSKI (8).

must have been built about the end of the later Stone Age and after the *Litorina* subsidence (33, 34). On the other hand, RAMANN (28) and POTONIÉ (25) concluded that the assumption of a change of climate is unnecessary, and that the horizon layer is determined by the physical characteristics of this type of peat. The double character of the sphagnum layer is accounted for by the gradual diminution of the water raised by capillarity during dry seasons in certain thicknesses of the peat material. "Die Sphagneen können dann nicht mehr aus den tieferen Schichten mit Wasser versorgt werden und sind auf jene Mengen angewiesen, die sie in ihrer wachsenden Schicht festzuhalten vermögen. Es werden dann zwei wasserreiche Lagen vorhanden sein, eine tiefliegende und die

Oberschicht, beide durch trockneren Torf getrennt." LESQUEREUX (16) also believed that peat deposits when checked by dryness form a parting layer between an old and a new bed of peat which takes on the shape of a dry layer.

With increasing study of the structural features of American peat deposits, correlations of various kinds will undoubtedly demand more consideration and will assume their basic importance. At present, however, it appears to be well founded to regard apparent structural climax layers as depending mainly upon the continuation of certain regional field conditions. It has already been suggested that the structural development of a peat deposit may fail to terminate on account of unfavorable local field conditions, and that various factors may inhibit a further development or may produce secondary stratigraphic features of varying character. There appears to be little doubt, however, that whenever

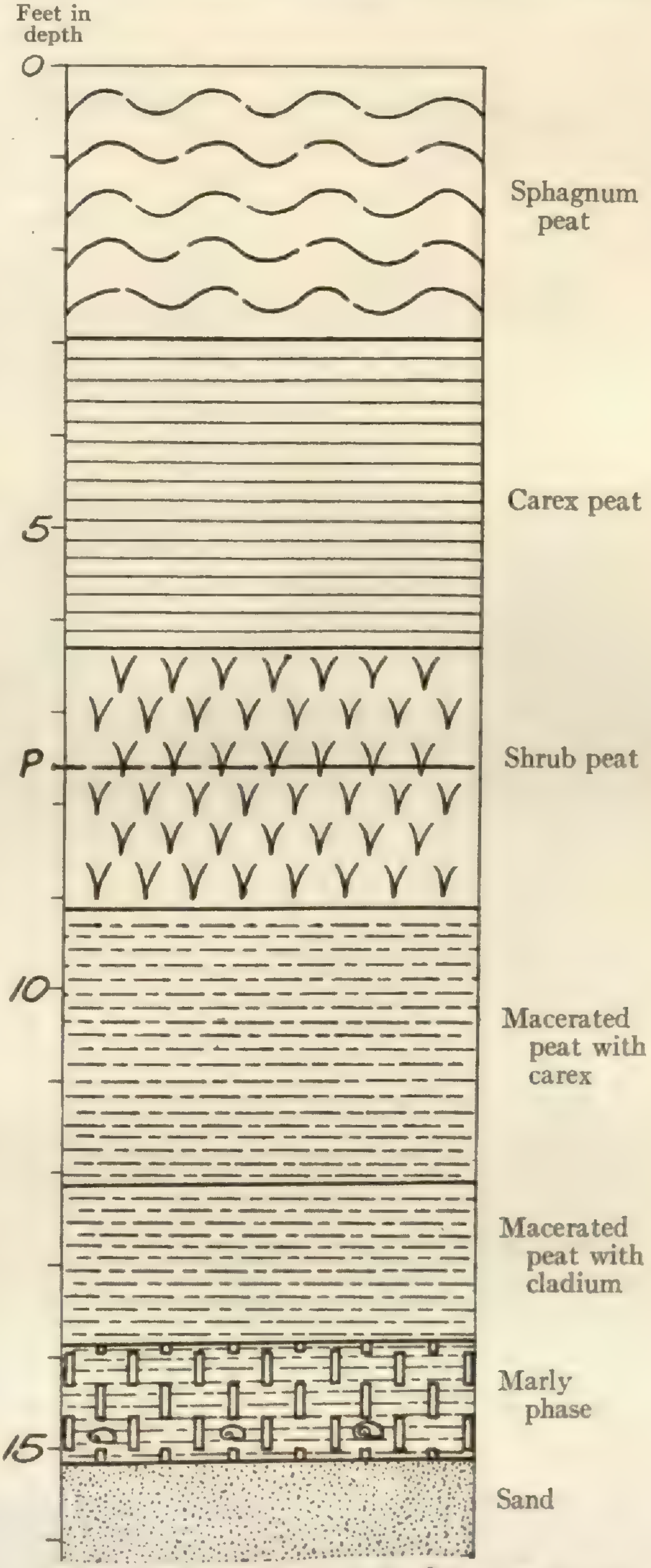

FIG. 6.—Profile section of the Åsle peat deposit, Hornborgasjön, Sweden; after SANDEGREN (31).

the movement of plant populations continues, either through a further change in habitat or in the development of new plant communities, the climatic vegetation unit is also the climax in the stratigraphic sequence of peat materials (figs. 5–7).

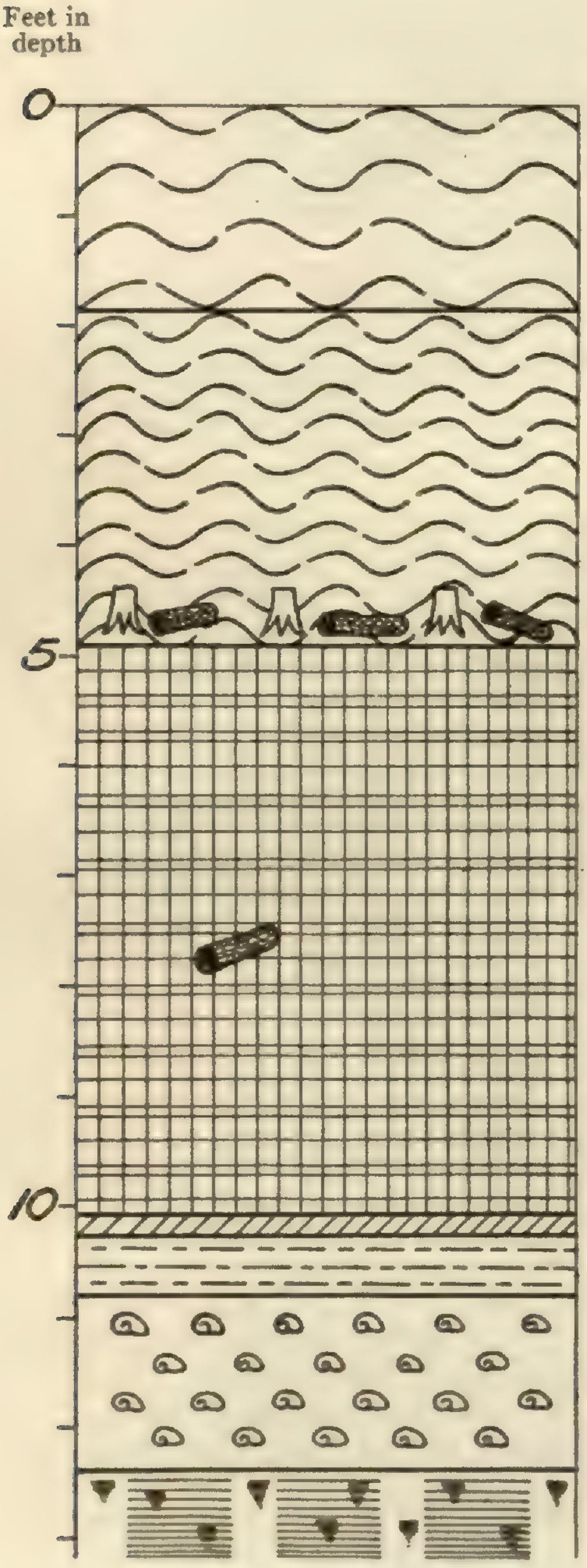

FIG. 7.—Generalized section of peat deposit of Lonsdale lacustrine moors, England; after RANKIN (29).

EVIDENCE OF CLIMATIC CHANGES IN OHIO

Peat investigations are still an unspecialized field in which the interrelation of climate, geology, and vegetation plays the paramount rôle. Whether in the service of science or of agriculture and other industries, the peat-land problem comprehends all the complex correlations of plants and their habitat; hence it should also furnish a historical perspective and the points of departure which lead to past relations. It would be presumptuous at this time to attempt to draw a parallel between the climatic changes recorded in the different peat deposits of this country. A reciprocal relation can scarcely be discovered, even in a general way, from only the few and incomplete records, and yet, although tentative, a short statement descriptive of the preliminary results obtained

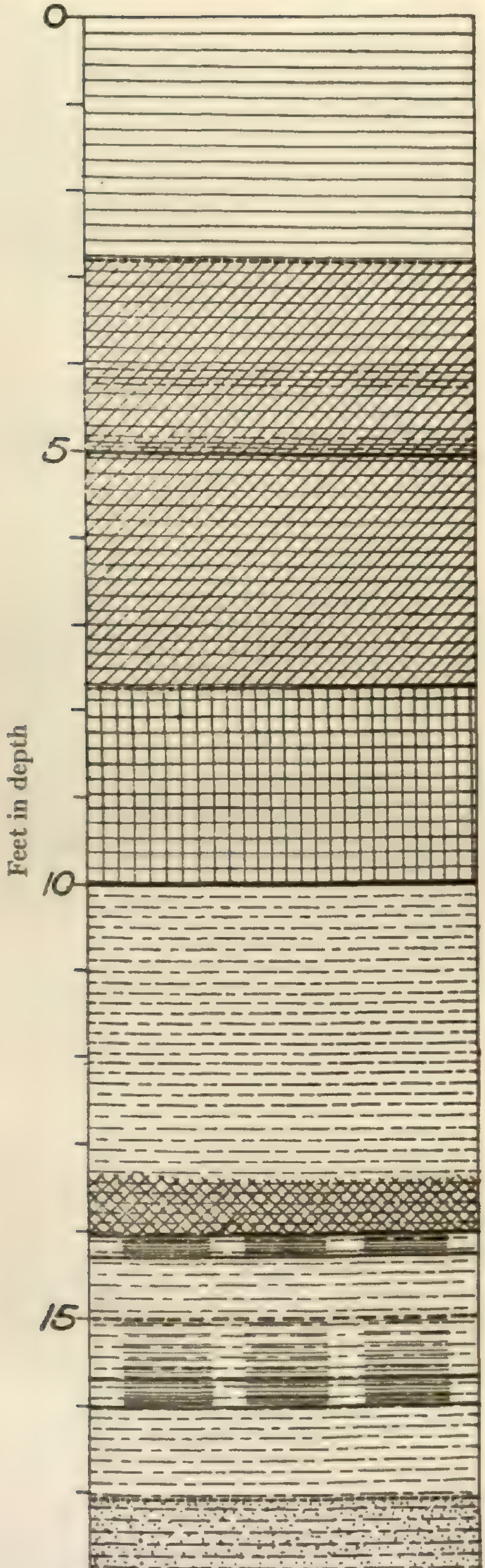

FIG. 8.—Section of peat deposit showing layers of peat material in deposit at Canton, Stark County, Ohio; elevation 1104 feet a.t.; sounding taken 300 feet south of 12th str., N.W., west side of N.O.T. car line.

may be of interest. A full correlation can be reached only by repeated efforts of this kind. Emphasis will necessarily fall upon glacial formations, because they are both an effect of climatic fluctuations and a cause in the age relation of peat deposits. The general microscopic analysis of the plant remains is reserved for a more comprehensive paper to follow.

With regard to the order of age, from older to younger, it is advantageous to compare briefly a few peat deposits (figs. 8, 10, 12) located between Canton and Cleveland, Ohio. In this part of Ohio several of the great morainic systems of the Wisconsin stage of glaciation are closely crowded together. They extend from Canton northward as a massive (interlobate) belt, and show nearly all the advantages of individual distinction

without involving the complications which occur in the broader intermorainic tracts as a result of subsequent changes in drainage. The general physical features of this area have already been described (4). An extended account and delineation of the geology and soils has been given by LEVERETT (17) and in the field operations of the United States Bureau of Soils (20, 21). A diagrammatic representation of the successive positions of the ice border and the location of the peat deposits is given in fig. 1.

The Canton peat deposit (fig. 8), like the Buckeye Lake deposit (fig. 3) farther southwest, is among the first and the oldest in Ohio. In both the basal layers of macerated plant remains represent accumulations of peat which probably began while the ice border was receding from its maximum position across Illinois, Indiana, and Ohio to about the limits of the Bloomington group of moraines. The growth of peat-forming vegetation in these two deposits followed soon after the recession of the ice sheet, before the drift had become drained by development of valleys on it.

Two seams of clay in the Canton deposit are noteworthy. Their positions indicate that the early period of peat formation was at least twice marked by climatic disturbances. The presence of the two layers of clay between layers of macerated types of peat seems to show that the ice readvanced to near this point into territory that had been laid bare following the maximum extension of the glaciers. In these states the western end of the Bloomington group of moraines not only overrides the weaker ridges of the Champaign moraines, but also extends into the ground occupied by the Shelbyville morainic system which was formed at the culmination of the Wisconsin stage of glaciation. The clay was probably deposited along the border of the ice mass by the same agencies that contributed the coarser material at the margin of the moraine, while further out, in the water basins, sand and finally clay were left. The deposition of clay may have taken place chiefly during the retreat of the ice front, when climatic conditions had become much warmer. It is not improbable that these clay seams represent the loess material which is known to cap the earlier morainic systems of the Wisconsin drift. Much of the material from the loess covered plains may have been carried

up by strong winds, forming at first a surface coating upon the ice at the time the moraines were developing. The effect of possible meteorological changes over wide areas, such as PENCK and others have worked out, must be borne in mind. The shifting of all climatic zones southward (**26**), caused by the general lowering of the temperature during the Ice Age and the depression of sea-level, points to the probability of this area as part of a relatively windy arid belt. After the ice had melted back some distance, the inorganic material may thus have come to be contributed to the peat deposit.

The basal layer of macerated peat is somewhat silty, and has a rather aged appearance. It is assumed, provisionally, to have been formed during the first or Shelbyville period of deglaciation. The layer of plant remains found overlying the basal peat has a much fresher aspect, but the organic débris in both of the lower layers of peat seems to fall short of reaching the greater variety of plant fragments which occurs in the succeeding layers. Further study of a microscopic nature, however, is necessary to establish fully the character of the plant remains from each of these glacial substages. The strongest evidence of an interval between the formation of the basal macerated peat and of the overlying layer of macerated plant remains is found perhaps in a comparison of the character and amount of the uppermost seam of clay. This clay seam is much more sharply terminated than the lower one, and it is also worth noting that the upper thickness of the clay stratum is compact and relatively free from plant remains. Whether or not the evidence thus far at hand favors the view that the seam of clay is derived from wind blown loess rather than from drift, or differences in the strength of the outwash, of considerably greater significance is the fact that the upper mineral layer constitutes a distinct break in peat formation. The cause of this break in the succession of peat materials must evidently have been a change from colder climatic conditions, from a more or less notable readvance, and a renewed aggression of the ice sheet.

Apparently the climate was undergoing amelioration at the time, probably giving rise also to a lower water table. That such oscillations have occurred is evident from the work of LEVERETT

and others. A certain degree of aridity seems to have prevailed, not only during the withdrawal of the ice, but up to the period which resulted in the formation of the Bloomington morainic system. The Bloomington period of peat formation was stopped by the upper seam of clay. This suggested correlation appears to be correct, for the upper clay layer can be connected closely with that part of the Valparaiso-Kalamazoo-Mississinawa morainic system which passes northeast of Canton through Portage County. The principle members of this group of moraines show west of here a marked differentiation of glacial lobes and a shifting of the lines of axial ice movements. The glaciers, as shown by the studies particularly of LEVERETT and others, encroached again over the surface of land that had been vacated by the earlier recession of the ice border. This readvance, the limits of which are marked by a morainal belt reaching from eastern Illinois and extending northward into Michigan to the vicinity of Kalamazoo and Battle Creek, has usually been designated the late Wisconsin stage. It covers a time of drift deposition reaching to the series of generally weak moraines which are included in the Lake Border-Defiance system.

During the time which elapsed while this ice front receded, and which may tentatively be called the Mississinawa glacial substage, the third tier of macerated peat was formed and probably also some of the superposed layers of fibrous plant remains. There is hardly any feature in the structure of the Canton deposit so conspicuous as the fibrous layers of peat, which rest on and in places grade into the underlying third basal bed of macerated organic material. The *Carex* and *Phragmites* plant populations, from which these layers of relatively coarse fibrous peat are derived, appear to have grown at ground water levels much lower than those which prevailed at later glacial substages. The uppermost beds of fibrous peat of more recent development contain an admixture of aquatic plant débris. They do not represent in their texture the features which would be characteristic of a gradual decrease in available ground water coincident with the closing of water basins by vegetation.

In the absence of more definite correlations, these three primary series of peat layers, namely, the several basal layers of macerated plant remains, the middle bed of coarsely fibrous peat, and the

upper layers of partly fibrous plant components, might be interpreted as representing three great changes in water level. They

FIG. 9.—Location of peat deposit near Kent, Portage County, Ohio, and of sounding illustrated in fig. 10; scale, 1 inch = 1 mile (2.5 cm. = 1.6 km.).

may correspond, therefore, to three climatic stages that left their traces in the structure of the Canton deposit. From this it would seem that a comparatively warm period with moderately humid

conditions must have been preceded and followed by two comparatively cool periods, characterized by changes between drought and wetness greater in degree than seasonal variations. For this interpretation, however, a series of various facts is doubtless required. A consideration of the structural appearance of the deposits in line north of Canton should give more adequate evidence of such alterations. They represent in part a contemporaneous and later age of peat formation which should bring into clearer perspective the probable climatic conditions during and after the close of the third glacial substage.

The recession of the ice front marked by the Valparaiso-Kalamazoo-Mississinawa morainic system to near the border of the Huron and Erie basins initiated the development of the Kent (fig. 10) and the Mantua peat deposits (fig. 12) in the order named. An examination of the profile sections suggests a long interval of peat accumulation. In about the middle of the Kent deposit there is evidence that here also an unusual disturbance had affected the course of peat formation, and that a well marked climatic change had occurred. The position of the layer of forest peat in the Kent deposit suggests that the change is contemporaneous with the deposition of the Lake Border-Defiance system.

At the bottom of the Kent deposit, overlying the bowlder clay, are shells of fresh water mollusks, and above them a layer of plant remains from aquatic vegetation. This is followed by macerated material, a part of which is distinctly gelatinous. The upper portion of the structureless débris merges into a layer of fibrous plant remains, showing that a mat of sedges and other marsh plants had covered the basin. When this stratum was formed, a mixed deciduous but predominantly coniferous forest appears to have been growing on the borders of the basin, which gradually encroached and finally occupied the entire peat-land area. The thickness of the layer of forest litter shows that the ground water level at that time was below the surface soil, and that the tract remained moderately moist for a considerable period of time.

It can scarcely be decided in the present state of investigation whether or not the end of the Mississinawa glacial substage was accompanied by a widespread dispersal of forest trees from south-

eastern portions of the United States. The first coarsely fibrous layers of *Carex-Phragmites* peat in the Canton deposit, and especially the middle forest bed of the Kent deposit, certainly have a suggestive feature of resemblance. Among land-laid peat deposits, the basal forest bed in the Kankakee Marsh near South Bend, Indiana, appears to indicate a corresponding time relationship, the climatic conditions of which favored forest associations more distinctly southern in range. Layers of coarse, fibrous peat material and of forest peat seem to offer the evidence of a prolonged warm period, during which migration of deciduous shrub and forest vegetation units might readily have taken place to areas considerably more northward than they are at the present time (14). From these facts there appears some support for the suggestion that the probable range in temperature and precipitation as well as the duration of this warm period made it possible for many trees

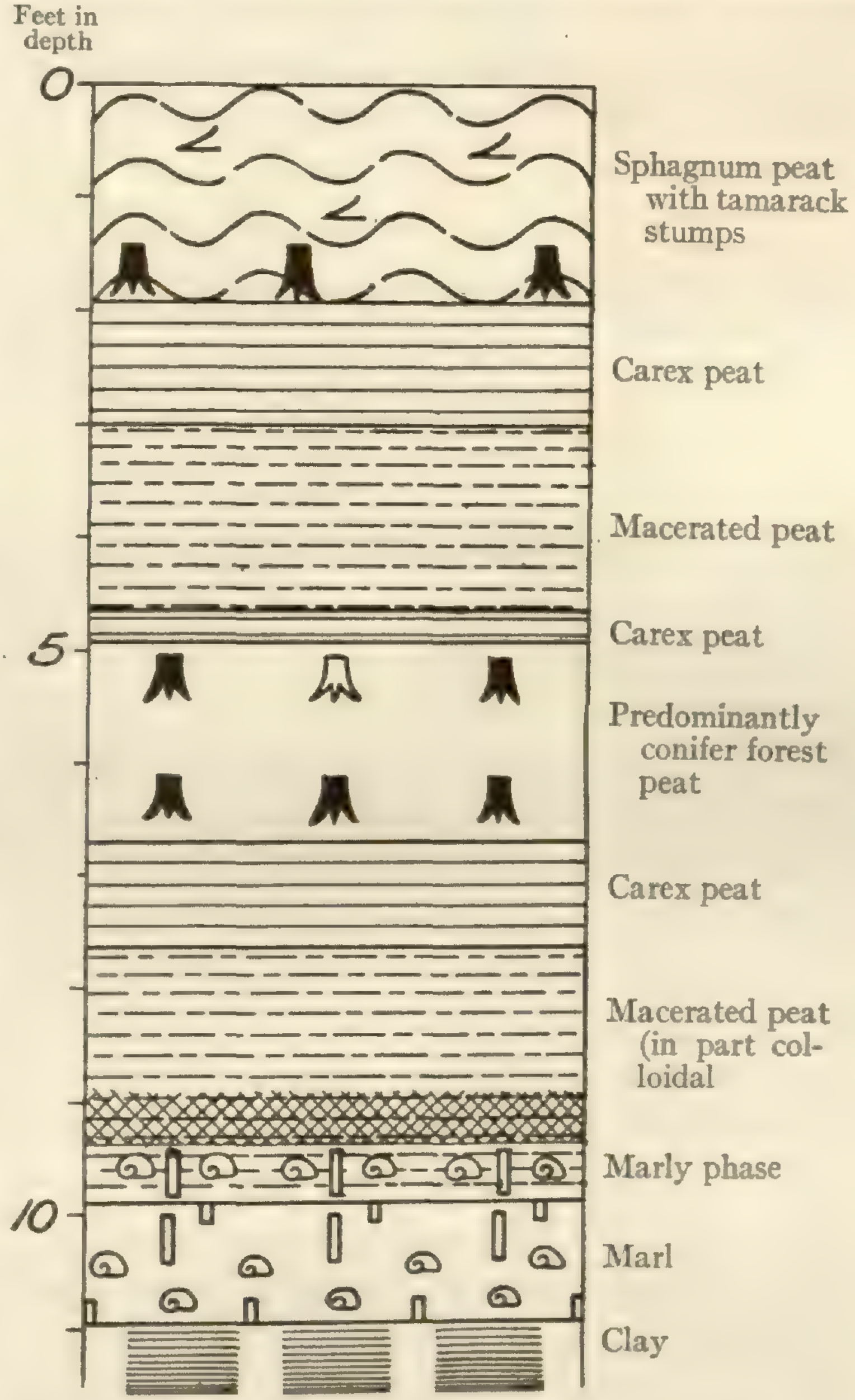

FIG. 10.—Cross-section showing structure of peat deposit near Kent, Portage County, Ohio; elevation 1028 feet a.t.; location of sounding indicated on map (fig. 9).

and shrubs to extend rapidly the limits of their distribution. It is unwise, however, to venture more, since at the present time definite stratigraphic and botanical data from peat deposits of northern states have not been exhaustively studied, nor have the investigations of the later quaternary deposits of the eastern and southern coastal states been carried to a point where they could be definitely correlated with the peat beds of this glacial substage.

There followed a wet period, during which the forest in the Kent deposit seems to have become submerged. The weight of the trees can scarcely have caused a sinking of the forest layer in this basin, since its depth is small and the underlying layers of peat material show no compression or disturbance. With the rise of the water table a layer of fibrous material from sedges and various other marsh plants began to accumulate above the forest stratum, but there soon followed a more rapid increase in the water level. The area became covered for a time with water.

The period of change recorded so conspicuously in the Kent. deposit appears to be associated with the Lake Border glacial substage. It is readily correlated with the time which elapsed when the front of the Erie lobe receded northward to the Port Huron morainic system. As the ice in its retreat uncovered the Ohio divide, inundation followed the escape of waters from the subsequent melting of the ice masses. No clay, however, entered into the formation of the peat deposit. The Lake Border moraines are practically free from loesslike silts, and apparently they were not strong enough to spread a seam of clay over this basin. When peat accumulation recommenced, there was again formed a layer of macerated material, followed by a fibrous type of peat from sedges, above which appears a stratum showing small twigs and branches of shrubs. Once more the area had become cool and dry, too severe perhaps for the free spreading of forests. Probably many tree species were again driven southward and replaced by more open vegetation, such as grassy marsh and shrubs. This cool period meliorated in severity rather rapidly and became sufficiently temperate for forests, for in the uppermost layer of peat are the remains of tamarack (*Larix* sp.). The stumps of the trees are standing in the peat itself. The present surface vegetation is a dense stand

of tamarack. In the partially wooded portion grow heaths such as *Cassandra* (*Chamaedaphne*) sp., *Vaccinium corymbosum*, and others, while the ground cover consists largely of sphagnum mosses with the cranberry and similar plants characteristic of sphagnum bogs. The southern portion of this tract is under cultivation.

Turning to the Canton peat deposit, it is interesting to note that the middle forest layer is wanting in this deep basin. The type of peat material of the period contemporaneous with the Kent middle forest layer consists of fibrous and relatively coarse plant remains from sedges and to some extent from reeds. The quantity of water must have diminished independently of the local alterations in the water table, for layers of a fibrous texture accumulate only under moderately moist conditions. The overlying peat stratum, on the other hand, is formed from *Hypnum* mosses and sedges, and has an admixture of macerated débris, clearly showing the advent of a cool period.

The succeeding layers in the Canton deposit show a gradual elimination of the *Hypnum* mosses as a peat forming component, and they also indicate a return of atmospheric conditions swinging toward a warmer climate. Before its cultivation the Canton area is reported to have been a marsh with the margins partly forested. Thus the uppermost layers of peat in the two deposits seem to show that during their later history, from the last glacial substage (the Port Huron time and the Lake Champlain period) to the present, the amelioration of climate has been relatively more steady than at any time since the culmination of the Wisconsin period. The lack of structural diversity is related probably to the distance of these deposits from the direct influence of the later glacial substages.

The beginning of the Mantua peat deposit (fig. 12), it is reasonable to infer, dates from the period of accumulation of *Hypnum* mosses in the Canton peat deposit and the submergence of the forest layer in the Kent deposit. In the Mantua deposit the uppermost layers similarly point to the supplanting of a cool by a more temperate period of climatic conditions, and to the migration of plants as an essential process in the sequence of peat materials. It is worth noting that the forest layer has the stumps of tamarack

(*Larix* sp.) in the lower portion of the stratum; while those of maple (*Acer* sp.), ash (*Fraxinus* sp.), and elm (*Ulmus* sp.) are found in the forest litter nearer the surface. The degree of natural drainage which established itself in time on the surface layers of this deposit determined, probably in large part, the character of the succeeding vegetation cover. Deciduous trees such as the red

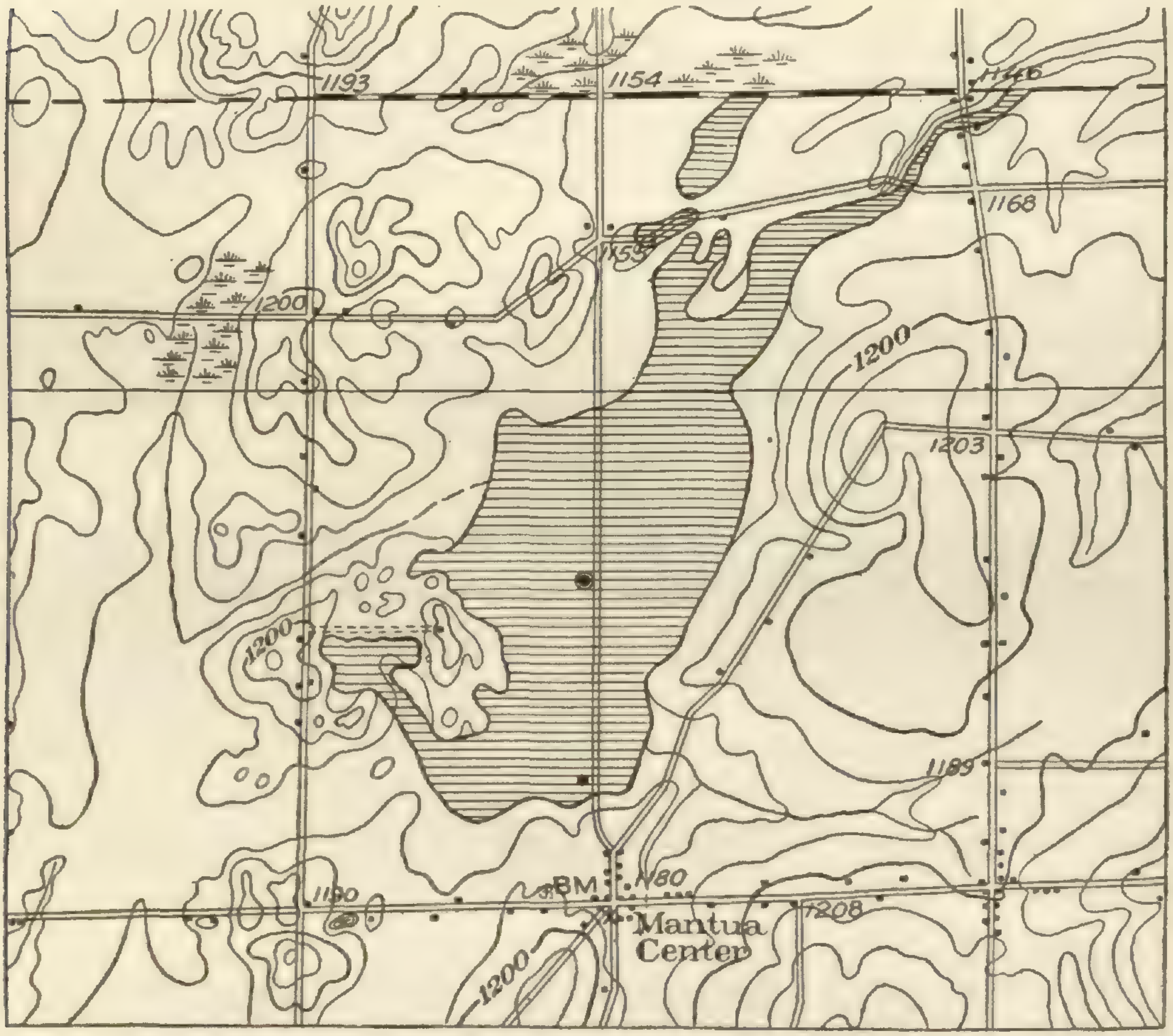

FIG. 11.—Location of peat deposit near Mantua, Portage County, Ohio, and of sounding on lot no. 9 illustrated in fig. 12; scale, 1 inch = 1 mile (2.5 cm. = 1.6 km.).

maple, black ash, and elm are still the dominant trees in the present surface vegetation of this tract of peat-land. Here again it is obvious that much is not yet clear about the major changes of climate until quite recent times, and that more extended and more critical field studies are required upon northern deposits which admit of ready comparison with the older peat accumulations. These questions of climatic changes from the later glacial sub-

stages to the present are critically important, for they bear radically on interpretations that have already been well supported in the countries of northern Europe.

It does not lie within the sphere of this paper to review the literature dealing with the probable causes which produced the glacial period or its climatic changes. These and other considerations are discussed fully by CLEMENTS (3), DOUGLASS (9), HUNTINGTON (11), and others. The only question is how far the types of peat material and their sequence in peat deposits may furnish evidence of climatic effects during the successively less extensive positions of the ice border. The facts given in this article seem to indicate at least three if not four major oscillations during which the climate fluctuated between warm and cold conditions, between periods of greater dryness and greater humidity.

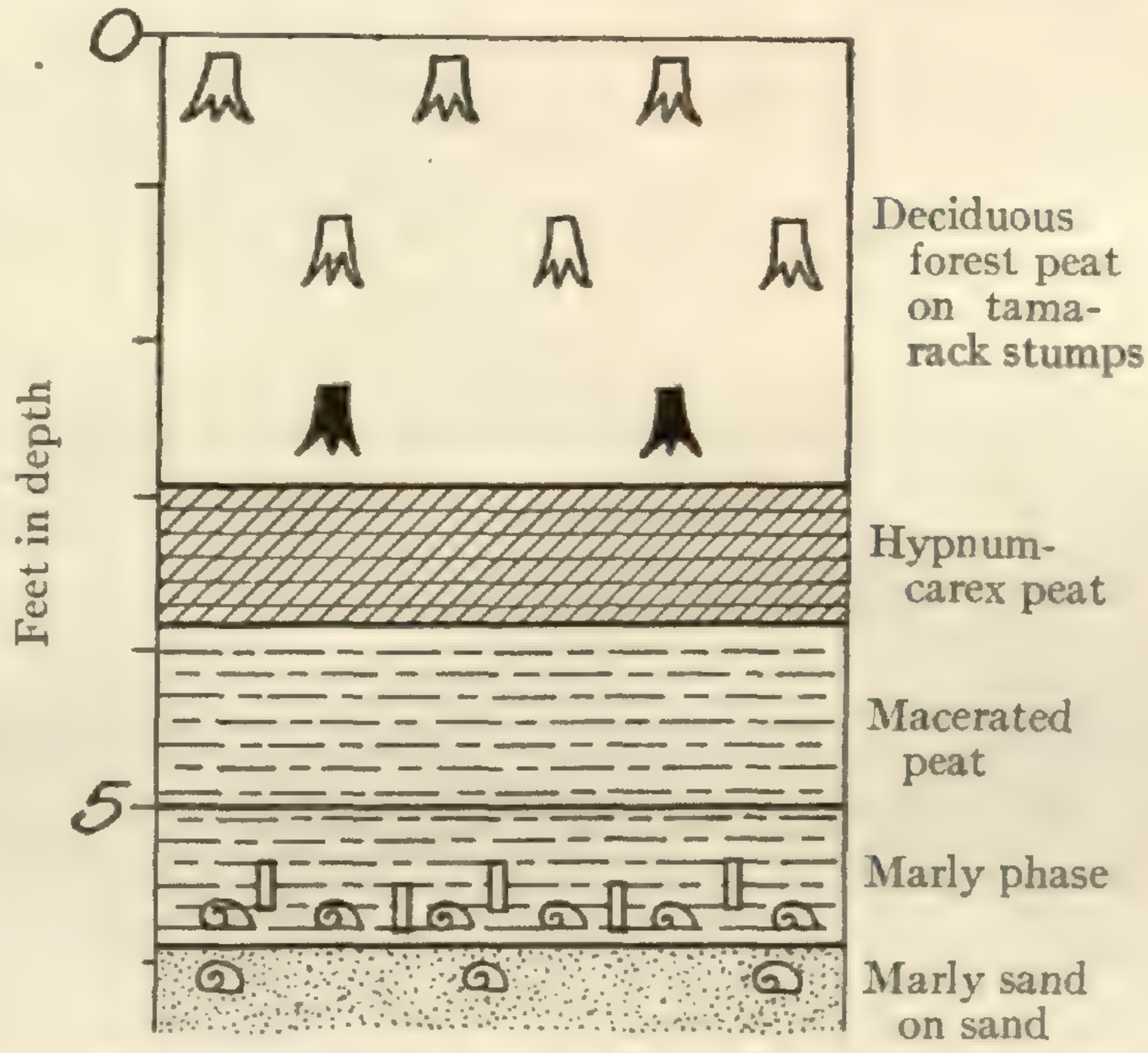

FIG. 12.—Profile section showing sequence of strata in peat deposit near Mantua, Portage County, Ohio; elevation 1155 feet a.t.; sounding on lot no. 9, west side of Center Road, as indicated on map (fig. 11).

Summarizing the climatic changes since the disappearance of the Wisconsin ice sheet in Ohio, the following may be stated tentatively: In the record of a few Ohio peat deposits an irregular series of changes can be traced, due to effects of climatic influences. Apparently twice a comparatively dry and cool period alternated with a relatively warm and humid period. After glaciation had reached its maximum extension, there followed two minor periods of recession of the ice field, a time during which a cool and dry climate bordered closely the glacial regions in this locality.

It was probably a period of winds, cooled from the ice sheet, and of loess deposition. The accumulation of drifted, wind-blown sand in the Kankakee, Indiana, area, portions of which were later covered by peat materials from a basal forest, may be referable to the first two glacial substages. In the general shifting of climatic belts the cold climate along the border of the retreating ice probably passed into dry windy conditions. On the exposed ground-till only marsh plants and low shrubs may have been the dominant plant population. This period of relative aridity in turn gave place to a second great advance of ice, the late Wisconsin, probably not of as great severity as the first, after which a prolonged warm and somewhat humid climate prevailed. This appears to have been the period of invasion and wide dispersal of forest trees from the south, and of a more northerly distribution of certain species than is now recorded for them. As to the end of the late glacial time, the climatic characteristics from the last glacial recessions to post glacial and present conditions stand as yet considerably ill defined. The evidences indicate periods during which the climatic zones shifted again somewhat. There appears to have been a return to cooler and drier climatic conditions, followed by a temperate and more humid period than exists at the present time in the same localities. The present period is probably approaching a climate of rising temperatures and (or) decreasing precipitation. The botanical data, however, are as yet insufficient to permit more definite conclusions, and they are wholly inadequate for drawing a parallel between the past climatic conditions of different countries.

The writer has had considerable hesitation in publishing the climatic correlations for the peat deposits of these great morainic systems. Although the interpretation accounts for a series of facts that are in need of being formulated, yet there might perhaps be another way of correlating the field observations. For this, however, the work of several years will doubtless be required. This preliminary paper may aid in the meantime a field of peat investigations to which BLYTT (**2**), WEBER (**35**), and others have been among the first contributors. With these major climatic fluctuations as a basis, chronological data of considerable value may perhaps be obtained by this method of peat investigations

for several sciences, including archaeological research. In its relation to the practical worker in peat-land problems it is hoped this paper will suggest the influence which structural differences in peat deposits necessarily exert upon a true estimate of the value of peat deposits and upon the progress of peat-land utilization, especially upon the plans, methods, and equipment which must be adopted to convert suitable areas into productive sources of national wealth.

UNITED STATES DEPARTMENT OF AGRICULTURE

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LIFE HISTORY OF CORALLINA OFFICINALIS VAR. MEDITERRANEA[1]

S. YAMANOUCHI

The group of red seaweeds known as the Cryptonemiales includes many species displaying a wide variety of form. The structure of the reproductive organs and the mode of reproduction found in this group cannot be ascertained adequately by the study of a single species. In order to distinguish the Cryptonemiales from the other groups of Florideae, the method of reproduction, as existing in *Dudresnaya*, has constantly been cited as characteristic and representative of the entire group; but it is merely characteristic of that genus. Moreover, our present knowledge of *Dudresnaya* is confined to its morphological features. In any systematic arrangement of the forms belonging to the ill-defined Cryptonemiales, *Corallina* should always be placed near the summit. This does not mean, however, that the structure of the reproductive organs and the mode of reproduction are more complicated than in other forms belonging to this group. About 30 years ago, SOLMS-LAUBACH published his original work on the structure of *Corallina*, but no cytological work was attempted, and the life history of the plant was not established. Consequently, a cytological study of *Corallina* was made from material secured at the Bay of Naples, Italy.

Origin of conceptacle

Generally the conceptacles are formed at the ends of branches of the thallus. The reproductive organs, which arise within the conceptacles, originate from these so-called disk cells which compose the central portion of the growing apex of each branch. The disk cells located at the periphery continue to divide and grow up around the reproductive organs, leaving only a small aperture or ostiole at the apex, thus forming the conceptacle. The three kinds

[1] Translated by CLARENCE C. BAUSMAN, assisted by C. CHIBA, from Bot. Mag. Tokyo **27**:279–285. *figs. 1–8.* 1913.

of reproductive organs (antheridia, carpogonia, and tetraspores) are produced in conceptacles on three different individuals.

Nuclear division in vegetative cells

Nuclear division is very common among any of the vegetative cells, but is most conspicuous among the actively growing (disk) cells at the ends of the branches. The cell structure varies more or less according to the position of the cell, but as a rule the nucleus occupies the central portion of the cell. One or sometimes two large vacuoles are present, and also many chromatophores. The cytoplasm seems to be distinctly alveolar in structure. The nucleus in the resting condition contains one and sometimes three or four irregular nucleoli. The chromatin material, during the resting condition of the nucleus, consists of small granules scattered throughout the karyolymph. Upon approaching the period of nuclear division, the nucleus slightly enlarges, and the chromatin, which up to this time was in the form of granules, increases in amount and finally becomes organized into a definite number of chromosomes. The male and female individuals possess twenty-four chromosomes, while the tetrasporic individuals have forty-eight. The contents of the nucleolus gradually become decreased with the formation of the chromosomes, and when the chromosomes have arranged themselves in an equatorial plate, the nucleolus has completely disappeared.

In the prophase a small, granular, centrosome-like body makes its appearance at each of the two poles of the nucleus in the cytoplasm near the nuclear membrane. Within the nucleus spindle fibers are soon formed, originating from the centrosome-like body. The alveoli of the cytoplasm immediately surrounding this centrosome-like body form branched astral rays. In later prophase the centrosome-like body becomes enlarged, and thus the centrosphere is produced.

In the metaphase the chromosomes divide, and when the two groups of daughter chromosomes reach the opposite poles, the daughter nuclei are soon formed. Up to and including the formation of the daughter nuclei, the centrosome-like body retains its characteristic form. With further growth of the daughter nuclei,

however, it gradually decreases in size, and at the approach of the resting stage of the nuclei it becomes unrecognizable. With the return of the period of nuclear division, the centrosome-like bodies reappear, as just described, at the opposite poles of the nucleus.

SWINGLE and others who studied *Sphacelaria* and *Stypocaulon* maintain that the centrosome or "central body" persists from one mitosis to another. HARPER appears to be of the same opinion, as a result of his work on *Lachnea* and *Phyllactinia*. As regards *Corallina*, the two centrosome-like bodies appear for the first time at the period of nuclear division, and gradually disappear with the formation of the daughter nuclei. Thus these structures are not permanent organs of the cell, but arise *de novo* at each mitosis to carry on the mechanism of nuclear division.

Formation of tetraspores

By normal cell division the disk cell divides into two portions, the upper portion becoming the tetraspore mother cell, while the lower portion becomes the stalk cell. The tetraspore mother cell in its growth assumes a clavate form, while its nucleus increases in size. At first the structure of the nucleus appears to be the same as that of the vegetative nucleus, but by the time the conceptacle has developed sufficiently to be recognized as such, the nucleus of the tetraspore mother cell enters upon the stage of synapsis. In *Corallina* the chromatin material is so scanty that a continuous spireme cannot be formed, but remains in two groups of small granules at the poles of the nucleus. When the synaptic period has passed, a centrosome-like body appears at each pole. In the metaphase a group of twenty-four bivalent chromosomes becomes arranged in an equatorial plate, and the paired chromosomes split longitudinally and separate into two groups. The first nuclear division, which is the heterotypic division, is soon followed by the second, or homotypic division. With the completion of the second division, there are formed four nuclei within the tetraspore mother cell, each of which possesses twenty-four univalent chromosomes. Later the tetraspore mother cell, by means of three cleavage furrows, becomes divided into four portions, each of which develops into a tetraspore containing one nucleus. The tetraspores then

escape from the conceptacle, float about freely in the water, and after becoming attached to a suitable substratum proceed to germinate.

Germination of tetraspores

The first nuclear division at the time of germination of the tetraspores shows twenty-four chromosomes. The same is true for the second and third divisions. With culture material the size of the plants obtained from germinating tetraspores was limited to thirteen cells. Throughout all these divisions there was no change as regards the number of chromosomes. The inference, therefore, is that such tetraspores, in nature, would give rise to sexual plants of normal size, possessing twenty-four chromosomes.

Formation of antheridium

The disk cell divides into two portions. The upper portion, which ultimately becomes the antheridium, is much smaller than the lower one, and is situated to one side of the latter. The two cells gradually become considerably elongated, the upper cell continuing to elongate until it finally attains a remarkable length. At the same time its nucleus divides, one daughter nucleus migrating to the extreme distal portion of the cell, while the other daughter nucleus remains in the lower portion. Just below the upper daughter nucleus a cell wall is formed, dividing the original upper cell into a very short terminal cell and a very long lower cell. The terminal cell becomes much enlarged and assumes a spherical form; the nucleus also enlarges greatly and occupies the larger portion of the cell. Thus the antheridium of *Corallina* is composed of a larger, spherical, terminal cell and a very much elongated, narrow, stalk cell. Later this spherical cell separates from the filiform stalk cell and functions as the spermatium. More than one antheridium may be formed from the same disk cell. The antheridial nuclei have constantly twenty-four chromosomes. The spermatium has a thin cell wall derived entirely from the mother cell, and when compared with other Florideae it is homologous with a unicellular antheridium. In 1911 SVEDELIUS, after having studied *Delesseria*, reported that the spermatium simply consists of the

naked protoplast discharged from the mother cell. I believe, however, that this would be disproved by a careful reinvestigation.

Formation of procarp

Each disk cell produces one carpogonial branch or procarp. The steps in the development of the procarp are as follows: The disk cell divides to form two cells, the upper one becoming the auxiliary cell and the lower one the stalk cell. The auxiliary cell then gives rise to a cell at one side of its exposed terminal portion, and then similarly to another cell at the other side. Thus two sister cells are produced from the auxiliary cell, situated side by side. Of these two cells, the first one formed has become greatly elongated by the time the second sister cell is formed. The nucleus of the older sister cell divides to form two nuclei; one nucleus remains in the enlarged basal region of the cell (carpogonium) and becomes the carpogonial nucleus, while the other one enters the hairlike upper portion of the cell (trichogyne) and functions as the trichogyne nucleus. The trichogyne is separated from the carpogonium by a constriction. The younger sister cell, which is usually provided with one nucleus, ceases to grow further at an early stage in its development, and simply remains as a non-functional structure beside the carpogonium formed by its older sister cell. Every one of the many disk cells, at the growing tips of the thallus branches, produces a procarp.

As just described, each procarp is composed of a stalk cell, auxiliary cell, carpogonium, and trichogyne, together with the small non-functional sister cell of the carpogonium. The structure of the procarp of *Corallina*, therefore, would seem to be simpler than that of other Florideae; yet in many Florideae the procarps are solitary, or, as in the case of *Ceramium*, two occur side by side. In *Corallina*, however, 60–70 or sometimes over 100 independent procarps occur in a group within the same conceptacle, and after fertilization, before the formation of the carpospores, they fuse with one another, resulting in the formation of one common structure.

Fertilization and formation of cystocarp

The trichogynes project above the surface of the conceptacle and are thus freely exposed to the sea water. A floating spermatium comes in contact with the apex of the trichogyne, adheres to

it, and discharges its contents into it. The trichogyne nucleus now begins to disintegrate. The spermatium nucleus proceeds downward, finally reaching the carpogonial nucleus, with which it fuses. At this time the auxiliary cell unites with the auxiliary cells of adjacent procarps, resulting in the formation of a large central cell within the conceptacle. The passage between this central cell and the carpogonium broadens. The sporophytic or fertilized carpogonial nucleus now passes into the large central cell. Since the sporophytic nuclei of all the procarps within the conceptacle migrate into this central cell, there are therefore over 100 sporophytic and also about the same number of gametophytic or auxiliary cell nuclei included in this common cytoplasm. The two kinds of nuclei found in the central cell differ as regards their structure. The sporophytic nuclei are usually large, rich in chromatin, and possess forty-eight chromosomes; the gametophytic nuclei are small, possess twenty-four chromosomes, and most of them gradually disintegrate.

Each sporophytic nucleus moves to the periphery of the central cell, where it divides to form two nuclei. One nucleus enters the cell which has been formed on the outer surface of the central cell, while the other nucleus remains inside the central cell. From the cell produced on the external surface of the central cell, a chain of cells is formed in basipetal sequence. These cells enlarge, become spherical, and when they have attained the size of tetraspores, gradually become constricted, separate, and finally escape from the conceptacle as carpospores.

Germination of carpospores

After the carpospores have escaped from the conceptacle, they begin to germinate within twenty-four hours. The first nuclear division is of the normal type and shows forty-eight chromosomes. The same is true of the second and third divisions. The sporelings continue to develop until the 17-celled stage is reached, all of the cell divisions being of the normal type and showing constantly forty-eight chromosomes.

Summary

1. The male and female plants of *Corallina* possess twenty-four chromosomes, while the tetrasporic plants have forty-eight chromosomes.

2. During the formation of tetraspores the forty-eight chromosomes become reduced to twenty-four. The tetraspores on germination show twenty-four chromosomes, and since twenty-four chromosomes appear in the vegetative mitoses of the sexual plants, the inference is that the latter arise from tetraspores.

3. The nuclei of the reproductive cells (spermatia and carpogonia) of the sexual plants possess twenty-four chromosomes. The sporophytic or fusion nucleus, as a result of fertilization, has forty-eight chromosomes. The sporophytic nuclei give rise by division to the carpospores, which also possess forty-eight chromosomes. The carpospores on germination show forty-eight chromosomes, and since forty-eight chromosomes appear in the vegetative mitoses of the tetrasporic plants, it is inferred that the tetrasporic plants originate from carpospores.

4. The male and female plants are gametophytic, while the tetrasporic plants are sporophytic. The sporophytic generation begins with the formation of the sporophytic or fusion nuclei, extends through the formation of the cystocarp and carpospores, and finally terminates with the formation of tetraspores on the tetrasporic plant. With the formation of the tetraspores, the gametophytic generation commences.

5. Thus *Corallina* is another clear example of the alternation of a sexual plant (gametophyte) with a tetrasporic plant (sporophyte), the cystocarp occurring as an early phase of the sporophytic generation.

TOKYO HIGHER NORMAL SCHOOL
TOKYO, JAPAN

INTRA-OVARIAL FRUITS IN CARICA PAPAYA

H. F. Bergman

(WITH SIX FIGURES)

An unusually interesting teratological phenomenon came to notice recently when in cutting open a fruit of papaya (*Carica Papaya* L.) five small secondary fruits were found within the seed cavity. Externally the fruit containing them was in no way

FIG. 1.—Papaya fruit cut longitudinally, showing seeds and secondary fruits in position; one secondary fruit turned over to show production of seeds; $\times\frac{2}{3}$.

different from any other specimen to indicate the presence of the secondary fruits. These were attached near the basal end of the fruit, growing out from the placenta and replacing the seeds. In addition to the four conspicuous fruits there was found also one very much smaller. Fig. 1 shows four of the inclosed fruits in situ. Only the style and stigma of the fourth, the smallest fruit, is visible.

Four of the five fruits consisted of an ovary surmounted by a sessile stigma. The ovary was not completely developed, in any case only a single carpel probably being represented. Each of the four larger inclosed fruits produced seeds. This may be seen from the figure, one of the fruits being placed to one side and turned over to show the incomplete development and the production of ovules. The stigmas, instead of being flattened and laciniate, as in normal fruits, were capitate, considerably swollen, spongy, and with tuberculate surface. The smallest fruit has a very small ovary, without ovules, the capitate stigma being borne on an elongated style (fig. 2). The inclosed fruits were yellow, being somewhat paler than normal fruits.

FIG. 2.—Sketch of small secondary fruit only partly visible in fig. 1; natural size.

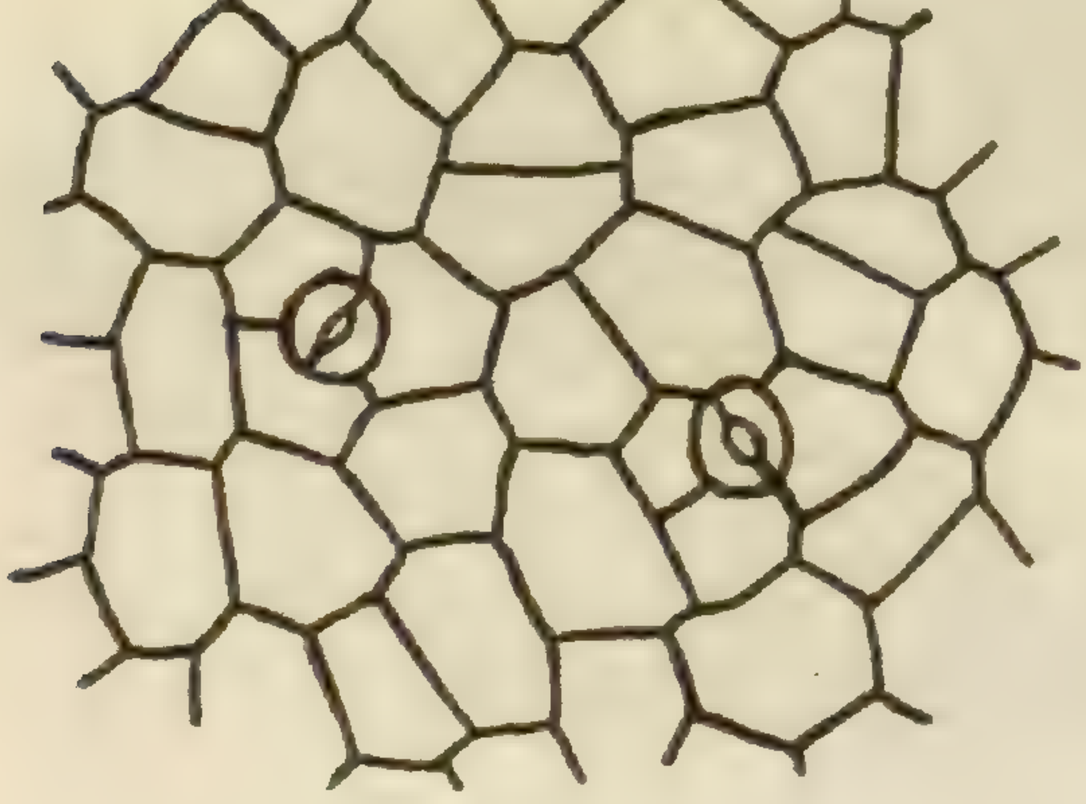


FIG. 3

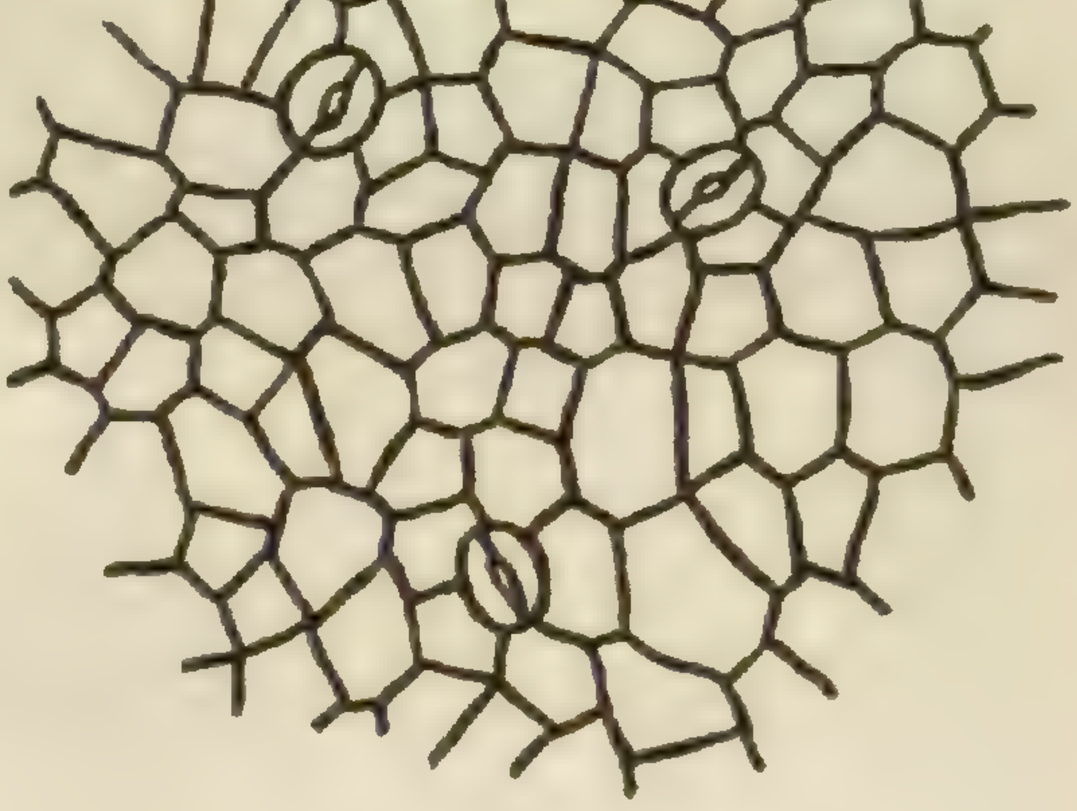


FIG. 4

FIGS. 3, 4.—Fig. 3, portion of epidermis of secondary fruit showing stomata; ×160; fig. 4, portion of epidermis of normal fruit showing stomata; ×160.

A microscopic examination of the epidermis of these fruits (fig. 3) showed it to be made up of cells similar in shape but somewhat larger than those of the epidermis of normal fruits (fig. 4). This similarity extended even to the presence of stomata. The guard cells were without chloroplasts. The only evident external difference in the epidermis of the inclosed fruits from that of normal fruits was in the absence of the coating of wax which the latter possesses in a marked degree. In cross-section the structure of the inclosed fruits resembles closely that of normal fruits, the

epidermis being thinner in the former. This and the absence of wax is probably due to the fact that they were not exposed. In all respects the seeds resemble those produced by a normal fruit. The embryo was found to be present in the several seeds examined, and to all appearances, so far as could be ascertained with a hand lens, was of normal form and size.

Some two or three months after finding these specimens, another set of intra-ovarial fruits of papaya was supplied to the writer through the kindness of Dr. L. O. KUNKEL, of the Hawaiian

FIG. 5.—Secondary fruits from seed cavity of papaya; slightly reduced

Sugar Planters' Experiment Station. These are shown in fig. 5. Only two of them are comparable in form and size to those shown in fig. 1. These two were rough surfaced, as may be seen from the illustration. The lower one of the three middle specimens shown in fig. 5 differed in being turbinate and smooth surfaced. In cross-section it was circular, without a seed cavity, but having a single vascular bundle near the center. All five fruits in this case were a very light cream color. No matured seeds were found, although the two larger ones had a placental surface with a few rudimentary ovules. The styles of the larger ones were filiform, tipped by a very small capitate stigma. An examination of the epidermis of

three of the five specimens showed it to be similar to that figured for the other specimens (fig. 3).

These intra-ovarial fruits, although they occur on the placentae, cannot with certainty be regarded as metamorphosed ovules. Instead it is more probable that they were produced from buds which developed adventitiously in places which would normally be occupied by ovules. The occurrence of adventitious formations within the ovary replacing ovules has been observed by several botanists. MASTERS[1] figures and describes a silique of *Cheiranthus*

FIG. 6.—Portion of papaya fruit showing secondary pistil as proliferation of stem axis; ×1.25.

Cheiri which contained an adventitious silique, replacing an ovule, within an ordinary silique, and also a grape which had another grape inside in the place of a seed. He also quotes and shows figures of a case described by BERKELEY[2] of a carnation in which the placentae bore both ovules and carpels. In this case transitional forms between the normal ovules and their carpellary transformations were found. Some of the carpels derived from ovules produced secondary ovules. MASTERS states that in the carnation

[1] MASTERS, M. T., Vegetable teratology. London. 1869.

[2] BERKELEY, M. J., Gardener's Chronicle, September 28, 1850 (p. 612).

specimens described by BERKELEY "the nucleus of the ovule was not developed." No transitional forms between ovules and secondary fruits such as were described by BERKELEY in the carnation were found in these papaya specimens.

The formation of secondary fruits within the ovary is evidently not uncommon in the papaya, and has been observed by many persons. It is said that in some instances the intra-ovarial fruits are exact models in miniature of the normal fruits. No information was obtained as to whether or not such fruits have a seed cavity with ovules or seeds.

An instance of the formation, in a different manner, of a secondary pistil within the seed cavity has also been observed. In this case the secondary fruit, instead of arising from the placenta in place of an ovule, occurred as a proliferation of the vascular axis which extends from the pedicel through the pericarp (fig. 6). The form of the pistil is not representative of the normal form in pistillate flowers, but is of the type that is to be found in a petunia or other similar flower. On cutting the ovary transversely it was found that no seed cavity was present. A single vascular strand was located in the center.

MASTERS refers to intra-carpellary prolification and states that "it occurs most frequently in plants having a free central placenta, though it is not confined to them, as it is recorded among Borragineae." No instance is cited, however, of a proliferation of the form here described.

UNIVERSITY OF HAWAII
HONOLULU

LEAVES OF CERTAIN AMARYLLIDS[1]

AGNES ARBER

(WITH EIGHT FIGURES)

In a previous memoir[2] attention has been drawn to the existence of leaves with a phyllodic type of anatomy among the Amaryllidaceae. In the present paper it is proposed to discuss certain special cases drawn from this family.

Leaf-anatomy of Narcissus

The foliage leaves of *Narcissus* consist typically of a linear limb (fig. 1, *l*) and a short sheathing base (*b*). In the very young leaves the sheath is relatively the more conspicuous organ, while the limb is scarcely developed. This relation is shown in fig. 2, drawn from a leaf which slightly exceeded 1 mm. in length. In *N. Tazetta* L. limbless sheathing leaves occur, in addition to foliage leaves in which both sheath and limb are developed. An examination has been made of the anatomy of the limb in the following species, representing the various sections of the genus:

SUBGENUS EUNARCISSUS

Section AJAX.—*N. Pseudo-narcissus* L.

Section GANYMEDES.—*N. triandrus* L.

Section QUELTIA.—*N. incomparabilis* Mill., *N. Jonquilla* L., *N. juncifolius* Req., *N. reflexus* Lois.

Section GENUINI.—*N. biflorus* Curt., *N. poeticus* L.

Section HERMIONE.—*N. Tazetta* L.

SUBGENUS CORBULARIA

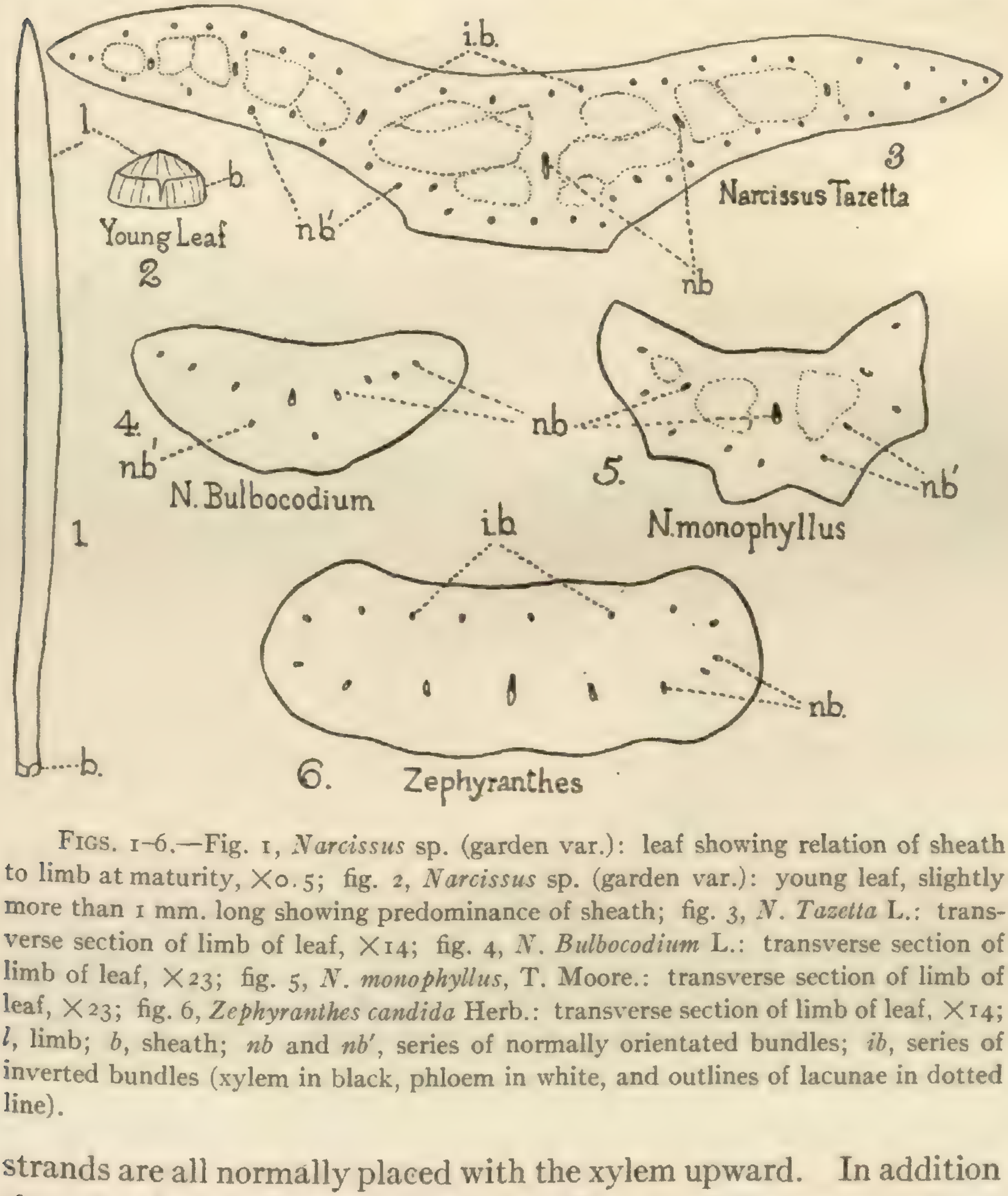
N. Bulbocodium L., *N. monophyllus* T. Moore.

Anatomy of the type interpreted as phyllodic[2] has been found in *Narcissus Pseudo-narcissus*, *N. triandrus*, *N. incomparabilis*,

[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher–Warr Studentship of the University of London.

[2] ARBER, AGNES, The phyllode theory of the monocotyledonous leaf, with special reference to anatomical evidence. Ann. Botany **32**:465–501. 1918.

N. Jonquilla, *N. biflorus*, *N. poeticus*, and *N. Tazetta;* that is, in at least one species from each of the five sections of the subgenus *Eunarcissus*. The leaf of *N. Tazetta* may be taken as a type (fig. 3). In this species there is a single series of main bundles lying roughly midway between the upper and lower epidermis (*nb*), and a series of smaller bundles lying near the lower epidermis (*nb′*). These

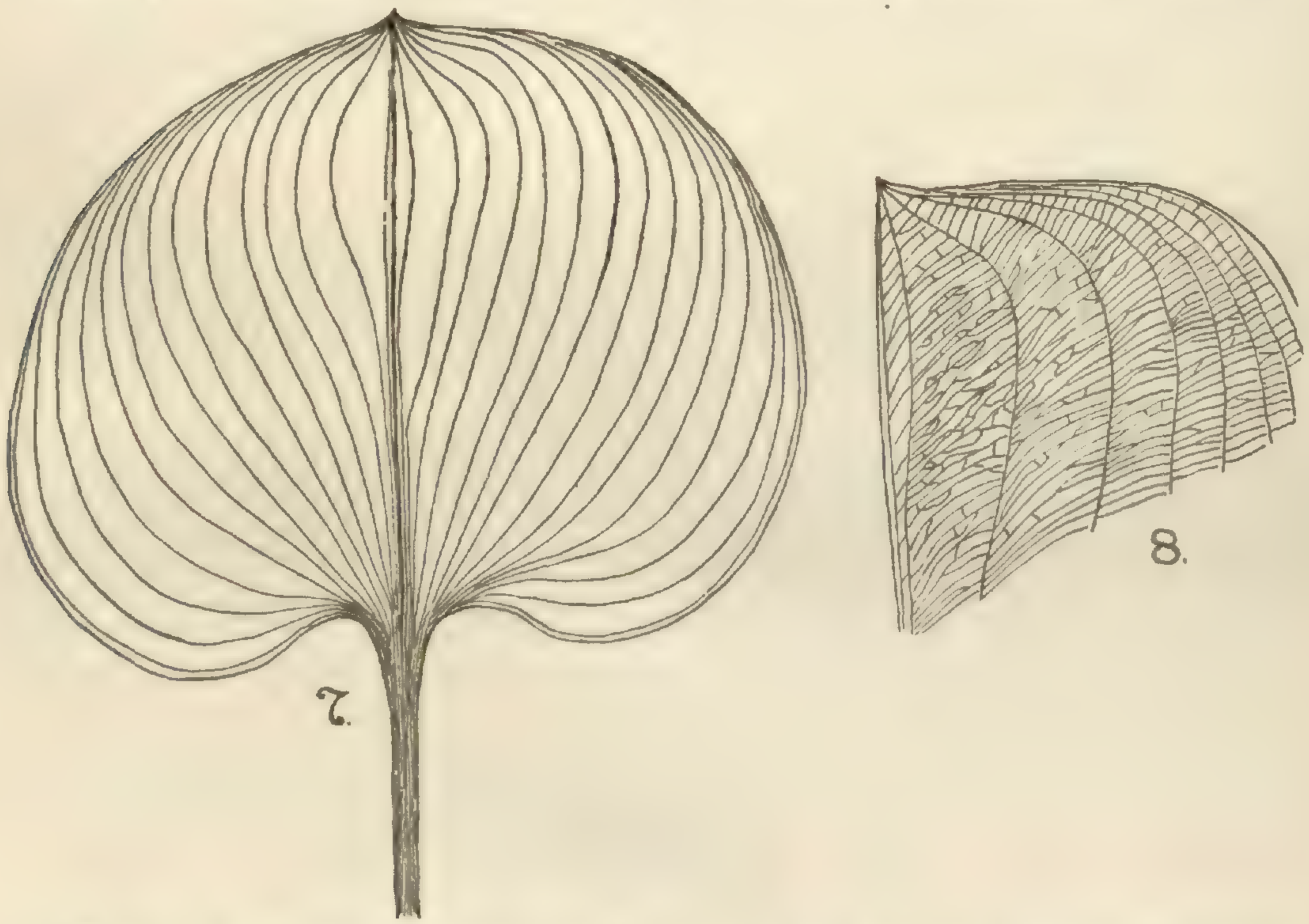


FIGS. 1–6.—Fig. 1, *Narcissus* sp. (garden var.): leaf showing relation of sheath to limb at maturity, ×0.5; fig. 2, *Narcissus* sp. (garden var.): young leaf, slightly more than 1 mm. long showing predominance of sheath; fig. 3, *N. Tazetta* L.: transverse section of limb of leaf, ×14; fig. 4, *N. Bulbocodium* L.: transverse section of limb of leaf, ×23; fig. 5, *N. monophyllus*, T. Moore.: transverse section of limb of leaf, ×23; fig. 6, *Zephyranthes candida* Herb.: transverse section of limb of leaf, ×14; *l*, limb; *b*, sheath; *nb* and *nb′*, series of normally orientated bundles; *ib*, series of inverted bundles (xylem in black, phloem in white, and outlines of lacunae in dotted line).

strands are all normally placed with the xylem upward. In addition there is a series of inverted bundles (*ib*) toward the upper surface. *N. triandrus* has a slender grooved leaf with peripheral bundles, whose xylem faces inward. The leaf anatomy of *N. Tazetta* and

N. triandrus may be compared with that of other Amaryllids in which inverted bundles occur toward the upper surface, as *Zephyranthes candida* Herb. (*Amaryllis nivea* Schult.) (fig. 6). In these cases the structure is interpreted as indicating that the limb is of a petiolar nature.

The only plants belonging to the subgenus *Eunarcissus* in which non-phyllodic anatomy has been found are *N. juncifolius* Req. and *N. reflexus* Lois.; in these all the bundles are normally orientated. This type of structure, however, although apparently rare in



FIGS. 7, 8.—*Eurycles sylvestris* Salisb.: fig. 7, leaf, petiole incompletely shown, ×0.25; fig. 8, small part of righthand side of leaf near apex, ×0.5.

Eunarcissus, is characteristic for the subgenus *Corbularia*. In both *N. Bulbocodium* (fig. 4) and *N. monophyllus* (fig. 5) only two series of bundles are found, both of which are normally orientated; the inverted series toward the ventral surface is absent.

The interest of the leaf anatomy of *Narcissus*, from the standpoint of the phyllode theory, lies in the fact that within the same genus there are examples of phyllodic anatomy (fig. 3), and of a reduced form of anatomy (figs. 4, 5) in which the loss of the inverted bundles results in a structure to some extent simulating that of a

true lamina. That the anatomical type shown in figs. 4 and 5 is indeed a reduction from that shown in fig. 3, and that the series should not be read in the reverse direction, are suggested by the general morphology of the subgenus *Corbularia*. The extreme corona development and the tendency to zygomorphy in the hoop-petticoat daffodil, as CHURCH[1] has suggested, point to its being a more advanced and specialized type than the various forms of *Eunarcissus*.

Pseudo-lamina of Eurycles

The leaf of *Eurycles sylvestris* Salisb. furnishes a very characteristic example of what has elsewhere[2] been described as the "pseudo-lamina" of the monocotyledon. The blade (fig. 7) is large. A herbarium specimen was measured in which it was 19 cm. long by 25.5 cm. wide. Fig. 7 shows that the primary skeletal system of this pseudo-lamina may well be interpreted as originating by the separation of the veins of the distal end of the petiole. The secondary and tertiary venation is also of interest from this point of view (fig. 8). A very large number of the secondary veins are unbranched and unconnected, and it is noticeable that the tertiary veins are extremely irregular; some pass from one secondary vein to another, some go from one secondary vein to a primary; while others leave a secondary vein, form a loop, and return to the vein whence they arose. The anomalous character of this venation seems not inconsistent with the view that the blade of the monocotyledon is an organ which is still at the experimental stage of its evolution from an expanded petiole.

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[3] CHURCH, A. H., Types of floral mechanism. Part I. Oxford. 1908.

A HOMOSPOROUS AMERICAN LEPIDOSTROBUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 283

JOHN M. COULTER AND W. J. G. LAND

Strobili of *Lepidodendron* so perfectly preserved that they can be sectioned and their minutest structures studied are common in European coalfields. Many of these strobili show heterospory, the megasporangia being at the base of the strobilus, the microsporangia above. The extensive literature of the subject is fully cited by SCOTT[1] and SEWARD,[2] and need not be repeated here. The extensive American coalfields, with but two exceptions, have yielded nothing but casts as yet. Perhaps the reason for this seeming scarcity of petrified material is that it has not been looked for carefully by competent observers.

In 1911 there came to this laboratory from Professor JOHN L. TILTON, of Simpson College, Indianola, Iowa, a well preserved fragment of a strobilus from the coalfields of Warren County, Iowa. This fragment, from above the middle of the strobilus, showed small spores, but of course nothing concerning heterospory could be determined. This specimen, the first American *Lepidostrobus* to be sectioned, was fully described by COULTER and LAND.[3] TILTON reexamined very carefully the place where the first fragment was found, and discovered several fragments of cones in a very good state of preservation, and evidently the same species as the first fragment. These he kindly sent to this laboratory. A few fairly well preserved stems of *Lepidodendron* also have been received from the coalfields of western Indiana. No cones were found, but it is evident that these fields will repay intelligent search.

Among the later fragments obtained from TILTON were four pieces which matched perfectly, showing clearly that they were

[1] SCOTT, D. H., Studies in fossil botany. London. 1909.

[2] SEWARD, A. C., Fossil plants. Cambridge. 1910.

[3] COULTER, J. M., and LAND, W. J. G., An American *Lepidostrobus*. BOT. GAZ. 51:449–453. *figs. 23.* 1911.

from the same strobilus, the tip being the only part missing. The restored strobilus was 22 cm. long and 5 cm. in diameter at the base. The structures were well preserved, with the exception of the axis, which is replaced by calcite and pyrites. The strobilus is mature; the sporangia have all opened and are empty excepting here and there a few spores. Enough sections were made to show its character, from the base to the broken tip, the general condition of the strobilus being almost exactly identical with that of the fragment described by COULTER and LAND. There is no appreciable difference in size of any of the spores, both those in the basal sporangia and in the sporangia near the apex averaging 27 μ in diameter. It seems probable, therefore, that this particular species of *Lepidostrobus* is homosporous, although it is possible that the spores found in the basal sporangia entered through the dehiscence slits. It would seem almost impossible that, in such a well preserved and compact strobilus, all of the megaspores, if there were any, could have escaped. The real solution of the problem lies in the finding of younger strobili which have not yet shed any spores. Negative evidence, however probable, is never conclusive; but the evidence in the present case is so strong that it seems safe to infer that this species of *Lepidostrobus* is homosporous.

The form genus *Lepidostrobus* was originally established to include all of the strobili of *Lepidodendron*. Later it was found that all such strobili could not be included, even in a form genus, so that "true" *Lepidostrobus* is restricted to those strobili of *Lepidodendron* characterized by "the great radial elongation of the sporangium, and its attachment by a long and narrow insertion to the upper surface of the sporophyll-pedicel throughout its length."[4] The chief interest in connection with these strobili is the question of heterospory. If heterospory has been attained by all these forms, the origin of the homosporous Lycopodiales is left in the region of the unknown. Certain species of *Lepidostrobus* are known to be heterosporous, and all of them are suspected. In one well preserved specimen the microsporangia occur in the upper part of the strobilus and the megasporangia in the lower part, as in certain species of *Selaginella*. The inference has been that

[4] SCOTT, D. H., Studies in fossil botany. London. 1909.

all the species of *Lepidostrobus* have probably reached the level of *Selaginella* in this feature, or, in other words, that *Selaginella* is the modern representative of this group. In the *Lepidostrobus* form referred to, the microspores are 20μ in diameter, and the megaspores 800μ, so that there is no question as to the great differentiation in size. The discovery of a *Lepidostrobus*, therefore, which is evidently homosporous is worthy of record and consideration. If these old strobili included both homosporous and heterosporous forms, the history of the modern Lycopodiales would become simpler. It would also emphasize the independent origin of heterospory in lines which could by no possibility be related.

UNIVERSITY OF CHICAGO

CURRENT LITERATURE

BOOK REVIEWS

A textbook of botany

FRITCH and SALISBURY[1] have prepared a sequel to their *Introduction to the study of plants*. In the more elementary volume the microscopic details were omitted, and therefore the present volume supplies these details for those who wish to know more about plants. Although the anatomical structure and reproduction of plants are the main subjects, the authors have included enough physiology and ecology to relate structure to function, and to indicate the responses of structures. In connection with reproduction, also, there is a supplementary chapter on heredity and evolution. It is interesting to note that the authors have abandoned the old method of types, and have treated groups as a whole, which certainly results in a better conception of the organization of the plant kingdom. They have also developed the economic contact when appropriate, stating that the purpose is "to combat the frequent ignorance of botanical students with respect to the economic aspects of their subject." This tendency is developing strongly in all the sciences, and is to be commended as developing a more general appreciation of science, and also as helping to do away with the old artificial distinction between "pure" and "applied" science.

The first part of the volume, devoted to anatomy, is an excellent general presentation of the subject, and one that is needed. There has been a tendency in the more recent texts to deal chiefly with the anatomy of the reproductive structures, with perhaps some supplementary information in reference to vascular anatomy, omitting the numerous other structures that enter into the structure of plants. In the second part, the life histories of the great groups are considered in evolutionary sequence, relating the facts to conditions of living and to future progress.

The volume is well illustrated, and should prove to be a valuable addition to the botanical texts for English students.—J. M. C.

Principes de botanique

It is a gratification to botanists, long familiar with the work of CHODAT, to greet the third edition of his well known textbook.[2] The first edition

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[1] FRITCH, F. E., and SALISBURY, E. J., An introduction to the structure and reproduction of plants. 8vo. pp. viii+458. *figs. 230*. London: G. Bell & Sons. 1920.

[2] CHODAT, R., Principes de botanique. Troisième édition, revue et augmentée. pp. x+878. *figs. 921*. Genève: Édition Atar. 1921.

appeared in 1907, and the third was ready in 1914, but its publication was prevented by the war and the unfavorable conditions of printing. The author has included the more important recent results of investigation, presented in his very attractive style, and with abundant illustrations.

The organization of the subject is peculiar to CHODAT, and therefore the volume has a flavor of its own. The four general divisions of the subject are as follows: "Physiologie générale," "La cellule, les tissus," "Physiologie spéciale," and "Génétique." The chapter topics under this general organization are often unusual. For example, practically everything usually treated under morphology, with the exception of anatomy, is presented under "special physiology," the evident suggestion being that structures are only significant in connection with their functions.

It is unusual for a book of nearly 900 pages to contain only ten chapters, and the subjects are suggestive of the organization. They are as follows: under general physiology, "Constitution de la matière vivante" and "Captation et transformation de l'énergie"; under the cell and tissues, "La cellule," "Organogénie," and "Anatomie"; under special physiology, "Fonctions de circulation et d'élaboration," "Fonctions de relation," and "Reproduction"; under genetics, "Variations, hérédité," and "Conclusion" (theories of the origin of species). The volume closes with a brief classification of plants.—J. M. C.

MINOR NOTICES

Dictionary of botanical equivalents.—ARTSCHWAGER and SMILEY[3] have prepared a very convenient dictionary which gives accurate translations of technical terms which are not usually found in ordinary dictionaries. All technical terms have been omitted when the English equivalent would be practically a repetition of French and German terms of Latin or Greek origin. As the compilers state, it is "a practical hand-book, accurate within the limits set for it." The publishers have also provided interleaved blank pages, so that users of the volume may amplify the list. It will certainly prove a very convenient volume for the reader of French and German botanical literature, both in saving time and in insuring accuracy.—J. M. C.

NOTES FOR STUDENTS

Chlorophyll inheritance.—Considerable interest has always been focussed upon reported cases of non-Mendelian inheritance. For the most part these have later been explained satisfactorily on a Mendelian basis, so that at the present time the only clearly recognized cases of non-Mendelian inheritance

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[3] ARTSCHWAGER, ERNST, and SMILEY, EDWINA W., Dictionary of botanical equivalents (French-English, German-English). 16mo. pp. ii+137. Baltimore: Williams & Wilkins Co. 1920.

are certain types of chlorophyll inheritance. WINGE[4] makes a hopeful attempt to classify all known cases of chlorophyll inheritance according to the following scheme:

I. The characters are situated in the nucleus and show Mendelian segregation, self-colored green usually being dominant. To this class belong the well known *alniba*, *citrina*, *chlorina*, *variegata*, and *albomarginata* forms of *Melandrium*, *Antirrhinum*, *Pelargonium*, *Mirabilis*, *Urtica*, *Aquilegia*, *Lunaria* (CORRENS, BAUR, and others). The most complex case on record is one recently solved by LINDSTROM[5] in which the F_2 of a trihybrid gives the remarkable ratio of 36 green:9 virescent-white:7 yellow:12 white.

II. The characters are situated elsewhere than in the nucleus and do not show Mendelian inheritance. This class is further split up in the following interesting way: (*a*) The characters are transmitted by the plastids themselves. In this case plants which are endowed with both green and colorless plastids may, through the uneven distribution of plastids of the two types at cell division, give rise to pure green and pure white areas, really a "somatic segregation." Such areas, through seed, will breed true to their local character. There is one known case, the "mosaic" *Pelargonium zonale* of BAUR, in which plastids evidently accompany the male nucleus at fertilization, for here inheritance is bi-parental. In all other known cases no plastids accompany the male nucleus, and inheritance is strictly maternal. In this last group we find the *albomaculata* forms of *Mirabilis* (CORRENS), *Antirrhinum* (BAUR), and *Primula* (GREGORY).

(*b*) The characters are situated in the cytoplasm. In this case no thoroughgoing "somatic segregation" is possible. A "hybrid" plant, combining the character for normal chlorophyll development with the alternative character for chlorophyll deficiency, will have these characters well diffused and intermingled through the cytoplasm. At cell division there may result a somewhat uneven distribution of these effective elements in the cytoplasm, resulting in relatively "lighter" and "darker" areas on the plant. This process of "dilution," however, has never been known completely to "purify out" either of the elements. Pure albinos or pure green individuals are never produced by these "hybrids." Here too we find in one case, the *albomaculata* form of *Capsella* (IKENO), the male nucleus seems to be accompanied by some cytoplasm, since the chlorophyll inheritance is bi-parental. In the only other case on record, the *albomaculata* of *Humulus* (WINGE), the male evidently contributes no cytoplasm, for inheritance is strictly maternal.

This classification seems fairly satisfactory, but it involves some fundamentally different conceptions from those of CORRENS and BAUR. Probably

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[4] WINGE, O., On the non-Mendelian inheritance in variegated plants. Compt. Rend. Lab. Carlsberg **34**:1–20. *figs. 4*. 1919.

[5] LINDSTROM, E. W., Concerning the inheritance of green and yellow pigments in maize seedlings. Genetics **6**:91–110. 1921.

the most significant fact, and one about which there can no longer be any doubt, is that chlorophyll inheritance is sometimes Mendelian and sometimes non-Mendelian. Naturally this suggests that other types of characters also may be, at least in some cases, non-Mendelian in inheritance.—M. C. COULTER.

Plagiotropic shore plants.—From the results of experiments carried on largely with *Atriplex prostratum*, TURESSON[6] reached the conclusion that the external factor causing prostrate growth is intense illumination, but that the growth movements are really geotropic in their nature. Emphasis is placed upon the fact that there are apparently two distinct sorts of plagiotropy, the one resulting from congenital habits of growth, and the other from response to environmental conditions. At times a single species, such as the one under experiment, will prove to consist of two such forms.—GEO. D. FULLER.

Haustoria of Meliola.—Miss DOIDGE,[7] in continuation of her studies of South African Perisporiaceae, has examined the haustoria of *Meliola*, whose species occur chiefly on leaves and shoots of forest trees and shrubs. She determined that the species are true parasites, sending haustoria into the cells of the host, penetrating the cuticle and in some cases sclerenchyma cells. The species differ in the length and character of the penetrating filament.—J. M. C.

North American flora.—Part 2 of volume 32 includes a continuation of Rubiaceae by STANDLEY. The preceding part included 20 genera, to which the present part adds 41 more. Much the largest genera are *Bouvardia* with 30 species (12 new) and *Exostema* with 26 species (5 new). The remaining 5 new species are distributed among the smaller genera.—J. M. C.

Vegetation of Paraguay.—Continuing his report on the scientific results of a botanical expedition to Paraguay, CHODAT[8] discusses the Apocynoceae, Urticales, and Araceae observed and collected. A number of new species are described, and rather extensive notes are made on distribution and ecology.—GEO. D. FULLER.

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[6] TURESSON, GÖTE., The cause of plagiotropy in maritime shore plants. Lunds Univ. Arsskrift. N.F. Avd. 2. **16**:no. 2. pp. 32. *pls.* 2. 1919.

[7] DOIDGE, ETHEL M., South African Perisporiaceae. VI. The haustoria of the genera *Meliola* and *Irene*. Trans. Roy. Soc. S. Africa **9**:117–127. *figs.* 7. 1921.

[8] CHODAT, R., La végétation du Paraguay. Fasc. 3. Geneva. pp. 291–379. *figs.* 53. 1920.

VOLUME LXXII NUMBER 3

THE

BOTANICAL GAZETTE

SEPTEMBER 1921

EFFECT OF DIRECT CURRENT ON CELLS OF ROOT TIP OF CANADA FIELD PEA

HENRY F. A. MEIER

(WITH PLATES II, III, AND THREE FIGURES)

Introduction

EFFECT OF CURRENT ON LIVING STRUCTURES

The numerous researches to determine the effect of the electric current on plants may be divided into two general classes: first, those in which certain organs or entire plants were subjected to electricity, the effect being measured by increase or absence of growth; second, those in which the effect of the current on the individual protoplast formed the basis of study. Among those interested in problems of the second class, AMICI (1) as early as 1818 suggested, although without experimental evidence, that protoplasmic streaming was of electrical origin. Impressed by AMICI'S suggestion, and by the striking results of his own and DUTROCHET'S work on the relation of temperature to protoplasmic streaming, BECQUEREL (2) attempted to show that the direct electric current had the same effect on protoplasmic streaming as variations in temperature. He placed cells of *Chara* in a helix, some parallel and others at right angles to the direction of the electric current, using a battery of 10–30 elements, without in any way influencing the rate of flow. With stronger currents, making direct connection with the cells by means of platinum electrodes,

protoplasmic streaming was inhibited. After a time the flow was resumed. No disorganization of the cells occurred.

JÜRGENSEN (12) worked with *Vallisneria*, noting the effect of the direct current on protoplasmic rotation. He placed sections of leaves on a special object holder, in distilled water, with copper electrodes in contact with the ends of the section. The effect was observed with a microscope giving a magnification of 235–680 diameters. Using 2–4 Grove cells, the speed of rotation of protoplasm was decreased, and long continued exposure to such weak currents brought about an inhibition of the streaming. If the current was discontinued after slowing down the rotation, but before it had entirely stopped, the original speed of rotation was reacquired after a time. When rotation had completely stopped, even though the current was broken immediately, movement was never resumed. Stronger currents produced the same effect as the weaker currents in much less time, the current from 30 cells sufficing to produce immediate and permanent inhibition of rotation. If the current was continued, the protoplasm contracted and gradually migrated toward the end of the cell nearest the anode, where it formed a dense mass against the wall. At break of current this mass would rebound toward the opposite end of the cell. On reversing the direction of current the mass migrated toward the opposite end of the cell, that is, toward the now positive end. JÜRGENSEN regarded these phenomena as coordinate with results he had previously obtained with unorganized bodies.

DU BOIS-REYMOND (7) had previously published similar results, experimenting with starch grains in the cells of a section of living potato tuber. Movement toward the anode was observed, and, as in *Vallisneria* cells, a reversal of current brought the starch grains to the opposite wall.

KÜHNE (14), using the direct current on a plasmodium of a myxomycete, grown on the slide between platinum electrodes 4 mm. apart, reported nuclei moving toward the anode, the cytoplasm toward the cathode. In the cells of *Tradescantia* stamen hairs the entire cell contents moved toward the anode. The ends of the cells toward the cathode changed from their characteristic purple to green, and the opposite end changed to a light red color. KÜHNE

experimented further with the effect of the current on unicellular organisms. Using platinum electrodes, he passed a direct current through a drop of water containing *Actinosphaerium*. The first visible effect of a weak current was the contraction of the pseudopodia lying in the direction of the electrodes. If the current was continued or increased, the pseudopodia lying in the path of the current became vacuolated, the vacuoles on the periphery burst, and the protoplasm of the rounded central portion of the organism began to disintegrate on the side toward the anode. This continued until the whole organism was disintegrated. KÜHNE states that the phenomena described for *Actinosphaerium* hold in general for such forms.

VERWORN (21) repeated and verified KÜHNE'S observations, using non-polarizable electrodes and extending the work considerably, especially with reference to free-swimming protozoa. He found in *Paramoecium* and other free-swimming forms a shrinking at the end toward the anode and a swelling at the opposite end, which phenomenon he regards as illustrating a general tendency to increased contraction on the side toward the anode. He found that some protozoa migrate toward the anode, others toward the cathode.

CARLGREN (3) observed that in *Volvox* the long axis of the colony is placed parallel to the lines of the current, and that there is a movement toward the cathode. If the current is long continued, the colonies move away from the cathode and sometimes gather at the anode. The movement of the flagella on the side toward the anode was inhibited, that on the opposite side not affected. Similarly to VERWORN'S observation on *Paramoecium*, CARLGREN noticed in *Volvox* an anodal shrinking and cathodal swelling with migration of the colony-forming cells (gonidia) within fixed colonies toward the anode. It is interesting to note his further statement that strong currents produced shrinking and swelling on the sides toward the anode and cathode respectively in dead specimens. Furthermore, he produced this shrinking and swelling in dead *Paramoecia* and *Amoebae*. CARLGREN concludes that the physical effect of the current (electrophoresis) accounts for many of the supposed stimulation effects on free swimming forms, and that it plays a large part in electrotaxis.

DALE (6) experimented with the effect of the current on five different species of infusoria found parasitic in the intestine of the frog. The organisms were exposed to the current in various solutions: neutral isotonic saline, slightly acid, and slightly alkaline solutions (using litmus as indicator). The organisms were exposed to the current in a trough with unglazed earthenware sides about 1 cm. apart, mounted on a slide, the ends of the trough being made of sealing wax. Non-polarizable brush electrodes carried the current to the porous earthenware sides. The results were very interesting. In slightly alkaline solution the organisms migrated toward the anode; when in slightly acid solution, toward the cathode. It is true that not all of the five species examined were equally sensitive to the acid and alkali treatment; that is, it required longer treatment in the solutions for some species than for others in order to produce the same effect. In more concentrated salt solutions (good electrical conductors) the migration of the organisms was inhibited.

More recently LILLIE (15) exposed to the direct current various animal structures: isolated nuclei, nuclei of spermatozoa, small leucocytes, and nuclei from lymphoid tissue, also muscle cells teased out in sugar solution, red blood corpuscles, and larger forms of leucocytes. These were suspended in N/4 cane-sugar solution (iso-osmotic with physiological salt solution). In freshly drawn frog's blood, the majority of the red corpuscles moved slowly (at an average speed of 120–130 μ per minute) toward the anode, many showed no migration whatever, and a few moved toward the cathode. The minute lymphocytes moved more rapidly toward the anode at a speed of 1500 μ per minute. The medium sized leucocytes were usually slightly negative (moved toward the anode) or indifferent. The larger leucocytes, however, with more cytoplasm, were in almost all cases decidedly positive (moved toward the cathode). The nuclei obtained by teasing thymus gland and the heads of spermatozoa moved rapidly toward the anode. The rate of the latter was about 2.0 mm. per minute. LILLIE'S conclusion, that "the direction and speed of living cells and portions of tissues are chiefly dependent on the electrical characteristics of their constituent colloids," seems justified.

In 1914 HARDY (9) published a short note on the migration under influence of the direct current of the contents of cells of the onion root tip. His methods and results were briefly as follows. The roots were placed horizontally between non-polarizable electrodes, the final lead to the tissue being some of the fluid in which the roots had been growing. As to the density of current used and the time of exposure, the author states: "A field of 5–20 volts per cm. was established for from 1 to 10 minutes, when the root was instantly fixed in acetic-absolute. Strength of field to which the living matter was actually exposed cannot be calculated." The effect produced was uniform, and varied only with intensity of current and time of exposure. The nucleus was usually slightly drawn out from a sphere to an ellipsoid, with the long axis parallel to the direction of the current flow. The nucleus maintained its position in the middle of the cell. The cytoplasm collected usually at the end of the cell toward the cathode, although frequently condensed into an equatorial plate. Within the nucleus the bulk of the solids collected at the side toward the anode. The nucleolus usually migrated toward the anode. No influence was exerted on division figures, spindles and chromosomes showing no sign of orientation or displacement whatever.

A careful review of the literature of this type of work reveals that current intensity was seldom measured accurately, and in most cases even when measured the results are not always reproducible because the organisms or organs studied were usually mounted in water, which acts as a partial conductor of current (conductivity varying with quantity of liquid used, electrolytes present, temperature, etc.), and in such experiments it is impossible to determine what part of the current flowed through the plant and what part through the water.

EFFECT OF CURRENT ON PARTICLES SUSPENDED IN LIQUIDS

The fact has long been known that finely divided particles suspended in water or other poorly conducting media will migrate, if the electric current is passed through the liquid, toward one or the other of the electrodes. Suspensions in water of starch, particles of paper, earth, asbestos, finely divided gold and copper all

move toward the positive pole. The particles of methyl violet, magdala red, lead, and bismuth move toward the negative pole.

REUSS (**20**) of Moscow seems to have been the first to discover the phenomenon of electrical migration variously known as electrophoresis or cataphoresis. He found that when two poles of a battery are immersed in a liquid and separated by a membrane the liquid will move through the membrane toward one of the two electrodes, and consequently the levels on the two sides of the membrane will not be the same. He discovered furthermore that while water moved toward the cathode, particles of various substances suspended in the water moved toward the anode.

That not all liquids migrate toward the cathode was first announced by QUINCKE (**19**). Oil of turpentine and absolute ethyl alcohol "that contained an organic impurity" migrated toward the anode. That the nature of the containers plays a part in determining the direction of flow was shown by the fact that in a glass tube lined with sulphur, oil of turpentine changed about in its direction of flow and migrated toward the negative pole. Water, however, was uninfluenced by the sulphur-lined tube and migrated, as in glass, toward the negative pole. The inhibiting influence of electrolytes on the movement of particles in suspension in an electric field was discovered by JÜRGENSEN (**11**). Suspensions of carmine in solutions of sulphuric acid, copper sulphate, and sodium chloride gave no evidence of movement when subjected to the current. On dilution of the easily conducting solutions, the particles again responded to the current.

That water, when absorbed by a semisolid material, will migrate was shown by DU BOIS-REYMOND (**7**). Incidental to this work was the invention of the non-polarizable electrode, which consists essentially of a short glass tube plugged at one end by moist kaolin, or by a camel's hair brush. Above the kaolin the tube is filled with a solution of $ZnSO_4$ into which dips an amalgamated zinc rod which is connected with the source of current. The semisolid used by DU BOIS-REYMOND was a cylinder of egg albumin. The non-polarizable electrodes were brought in contact with the ends of the cylinder, and in passing the current the end in contact with the positive electrode developed a constriction a short distance from

the surface of contact with the clay. The constriction became hard to the touch, the remainder of the cylinder swelling somewhat. When the current was reversed, the constricted end became soft and enlarged and the opposite end became constricted.

In repeating a part of JÜRGENSEN'S work, QUINCKE discovered that not under all conditions do the particles in suspension move toward the positive pole. Starch grains in water in a glass tube, as well as particles of silk, cotton, and paper migrated toward the positive pole when suspended in water, and toward the negative pole when suspended in oil of turpentine. The theory in explanation of these phenomena of poorly conducting liquids migrating in one direction and suspended particles in the opposite direction under the influence of the electric current, was propounded by HELMHOLTZ (10). The fundamental assumption of this theory is that at the surface of contact of any suspended particle there exists a double electric layer. If the particle bears a negative charge, the layer of medium immediately surrounding it bears a positive charge. On passing the current a displacement of one system against the other takes place, the liquid particles migrating toward one pole, the suspended particles toward the opposite pole. How the charge originates is not explained.

An explanation of the movement in opposite directions of oil of turpentine and water, and substances suspended in them, was first suggested by COEHN (5). He showed that with reference to the sign of the charge of a solid in contact with a liquid, the substance with the greater dielectric constant is positive to the other substance. The dielectric constant of oil of turpentine is 2.23, that of glass 4–7 (according to composition), and that of water is 81. In agreement with this explanation, glass is positive in oil of turpentine and negative in water. Water has a much higher dielectric constant than most other substances, and, as we have seen, most substances are negatively charged in water.

Observations on electrophoresis in colloidal "solutions" or sols were published by PICTON and LINDER (18) in 1892. Such colloidal sols consist essentially of very finely divided ultra-microscopic particles suspended in a liquid. Since LINDER and PICTON'S publication the work has been much extended, and the conclusions seem

inevitable that each colloidal particle bears a surface charge, which in some cases is negative and others positive, as indicated by the characteristic differences in the migration of different colloids. Furthermore, the researches of LINDER and PICTON show that the sign of charge possessed by the particles in a hydrosol bears a definite relation to the chemical composition of the particles, acid particles bearing a positive charge and basic particles a negative charge.

PERRIN (17) extended the work and formulated the following rule: "In the absence of polyvalent radicals, all non-metallic substances become positive in liquids that are acidic and negative in liquids which are basic."

HARDY (8) found that colloidal particles of derived albumins "move with the negative" (that is, they bear a negative charge and move toward the positive electrode) "if the reaction of the fluid is alkaline, with the positive stream if the reaction is acid."

The present work was undertaken with the idea of determining in a more or less quantitative way the effect of the direct electric current on the protoplast, the actual amount of current flowing through the organ being under control at all times. The investigation consists of two phases: first, that of cytological effect produced; and second, the combining of two factors, time and current intensity, to produce death of the protoplasts.

Materials and methods

Young seedlings of *Pisum sativum* of the variety known as White Canada Field Pea were chosen as the best material for this work, although onion, lupine, and Scarlet Runner bean were tested and the same cellular phenomena produced. The pea seedlings were grown in moist sawdust in ordinary 8-inch flower pots. The seeds were soaked in water for 12–24 hours, when all those not of uniform size were discarded. The hulls were removed and the seeds planted in the moist sawdust with the radicles pointing downward. The pots were then placed in the greenhouse. This method, if the moisture conditions of the sawdust are correct, will produce uniform germination and seedlings with straight radicles. Two lots were planted each day, one in the morning and one in the

evening, giving a choice of between 200–400 seedlings per day. The pots of seedlings were carried to the laboratory where the experimental work was done. In subjecting the seedlings to the current the following method was employed. The seedlings were subjected to the current one at a time in a moist chamber (text fig. 1) made of plaster of Paris and consisting of a box 18 cm. high, 10 cm. wide, and 7 cm. deep with one side open. A slab of plaster of Paris, cast to fit closely, was set against the open side to serve as a door. Through the top of this chamber the two non-polarizable electrodes were inserted. These were made as described earlier, but in actual practice it was also found that inserting the copper conducting wires without the zinc and zinc sulphate gave no polarization within 30 minutes, and with frequent changes of the moist kaolin was regarded as entirely safe.

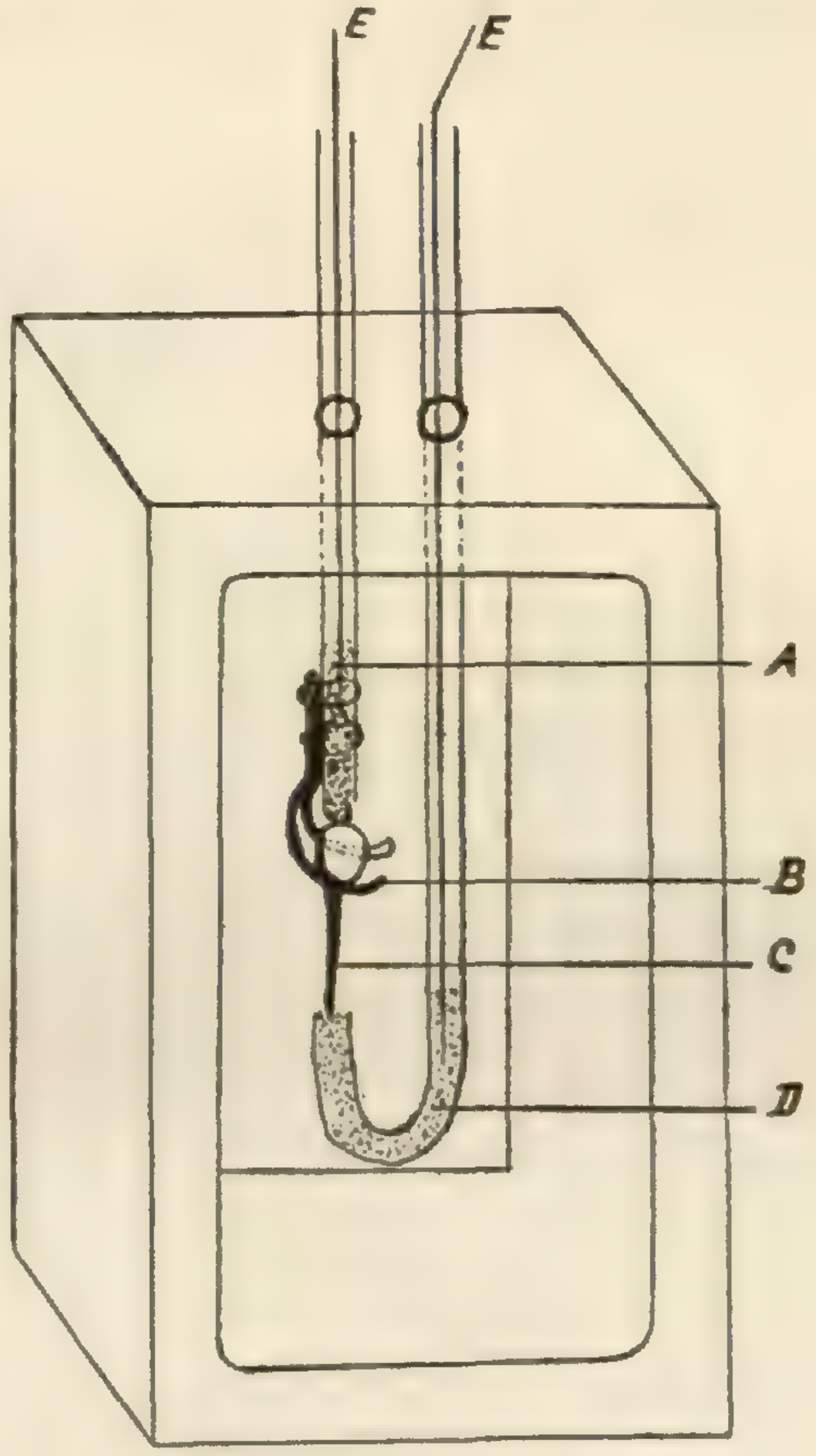

FIG. 1.—Moist chamber for exposing roots to current: E, E_1, wires leading to moist kaolin through glass tubes; A, D, kaolin in contact with extremes of seedling; C, root of seedling; B, glass fork for holding seedlings in position, and attached to glass tube of upper electrode by rubber bands.

Before setting up the electrodes, the moist chamber was placed in water for 20–30 minutes. This insured against the roots being exposed to a drying atmosphere while being exposed to the current. The arrangement of the conducting wires, together with resistances and measuring instruments, are diagrammed in text fig. 2. B and B^1, represented as binding posts in the diagram, consisted in reality of an ordinary lighting socket with key. From this socket a cord connected to an ordinary wall socket served as a source of current from the 110–120 volt direct current circuit. In some cases for added resistance a series plug with 4, 8, or 16 candle

power lamps was introduced at the wall socket. The current source in all cases, however, was the same. The sliding contact rheostat R has a resistance of 1770 ohms and a capacity of 0.45 amperes; while R^1 has a resistance of 464 ohms and a capacity of 1.2 amperes. The voltmeter V was attached to the binding posts C, C^1. The millivolt meter A (text fig. 2) was of Weston Electrical Instrument Company manufacture, with an upper range of 150 milliamperes and a lower range of 1.5 milliamperes. M represents the moist chamber.

The seedlings were carefully lifted from the sawdust, the adhering particles brushed off with a small camel's hair brush, the length measured with a small millimeter rule, and then by means of tweezers they were placed on the small glass fork which served as a

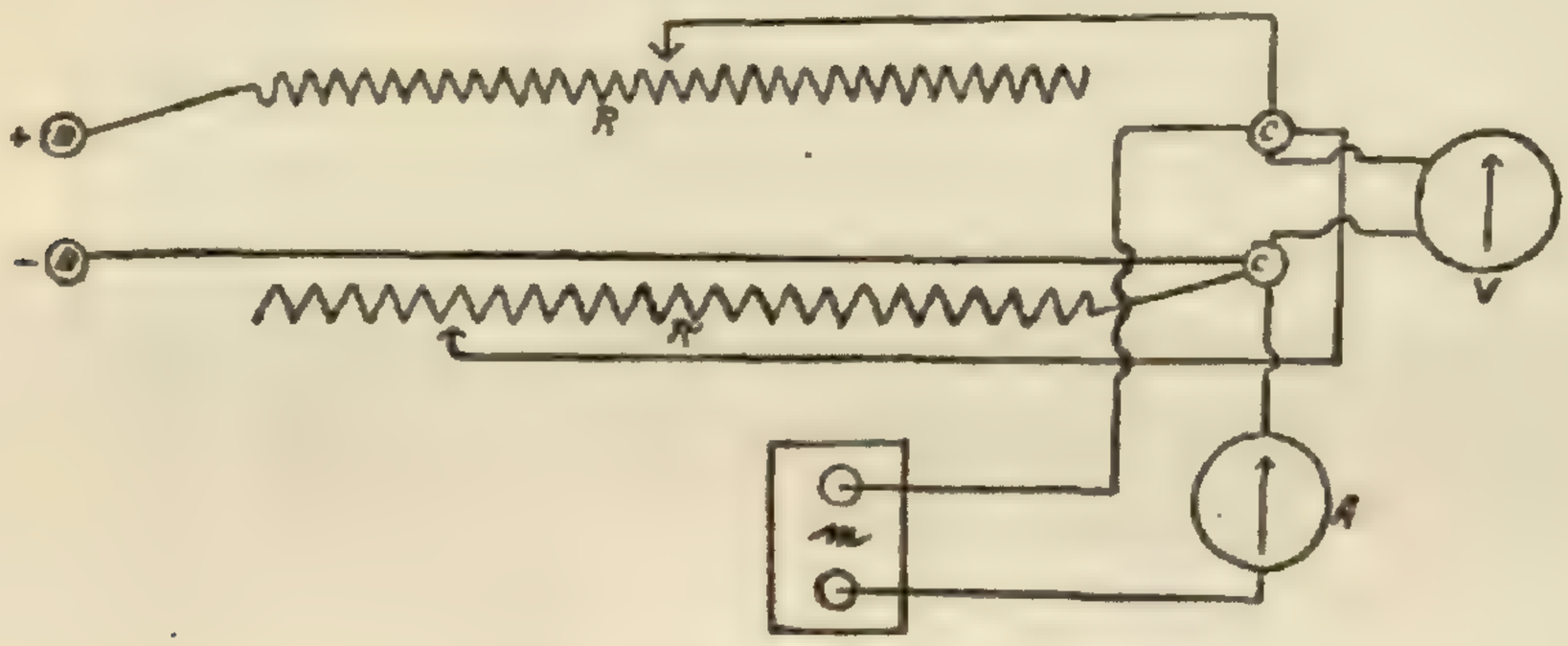

FIG. 2.—Apparatus and measuring instruments

support and which was attached to the upper electrode by means of a rubber band. The cotyledons of the seedling were now brought in good contact with the moist kaolin of the upper electrode, and the lower electrode (shaped like the letter J) moved upward so that the kaolin came in contact with not more than the first millimeter of root tip. The door was now placed in position and the current turned on with the key of the socket at B, B^1. The time of exposure was measured with a stop watch. During exposure voltage and amperage were read on the instruments.

For the cytological phase of the investigation the roots, immediately after exposure, were killed in Flemming's fluid. It should be stated that control seedlings were suspended in the moist chamber, in every case, near those being exposed to the current, and the roots killed and sectioned as the treated roots for comparison.

For that phase of the investigation relating to current intensity and time of exposure required to produce death, the seedlings were placed immediately after treatment in moist pine sawdust in glass battery jars. A stick the size of a lead pencil was pushed down next to the glass, the seedling placed in the opening thus made, and the sawdust carefully brought around the root. Figures on labels placed above each seedling served for identification when observation was again made, usually 24 hours after treatment. From 4–6 controls were placed in each jar of 12–15 treated seedlings.

All seedlings were carefully selected with special reference to length and diameter, those not corresponding to type being rejected. Only roots of 15–20 mm. length were used and of diameter as nearly uniform as examination without actual measurement could select. No practical means suggesting itself of measuring the diameter of the roots before exposure to current, the approximate diameter of the roots used was found as follows. Free-hand sections were made of 125 roots (carefully selected as if for treatment with the current), 3–4 mm. from the tip, the sections placed in a drop of water on a slide and diameter measured under the microscope with eyepiece micrometer. It was found that all fell within the following measurements: long diameter, 1.03–1.18 mm; short diameter, 0.84–0.96 mm. It will be seen from these dimensions that the roots were somewhat flattened. The cross-sectional area of the roots varied then from approximately 0.7 sq. mm. to 0.9 sq. mm. The diameter is an important factor, since on it depends the density of any given current intensity per unit area. It should be understood that these ranges are the extremes, the majority exhibiting no such variation as the extremes might indicate.

With the method described the actual current flowing through the tissue can be measured and read from the milliammeter, and calculated for the unit area. More or less just criticism is often made of the method of exposure to current of unicellular organisms in electrotactic experiments when the material to be examined is placed in liquid. The amount of current flowing through the organism depends therefore on whether or not the liquid surrounding it is a good conductor. If the liquid medium is a poor conductor, the current will pass in large measure through the organism;

if the opposite be true, very little if any of the current will pass through the organism. It is very evident that, since the conducting power of the medium is vastly increased by small amounts of electrolytes, the conductivity of the liquid is an ever-changing value. It is likewise evident that the same objection applies to roots in water through which the current is passing. Furthermore, the literature of electro-physiology is filled with references to current strength as weak, medium, strong, or with the mere statement of the number of cells used, with no statement of resistance in the circuit, so that actual current intensity cannot be determined, and difficulty is experienced in even approximating the conditions of the experiment. With the materials and methods just described these difficulties are avoided.

Observations

Killing effect of current

If a seedling with a root of 15–20 mm. in length and of 0.7–0.9 sq. mm. in cross-section is exposed in the manner described to a current of 0.3 milliampere, the following changes take place. In about thirty seconds the root begins to lose its normal color and becomes watery in appearance. If the current is continued for two minutes or longer, numerous very fine droplets of liquid appear over the surface 3–6 mm. from the tip. If the current is now stopped and the root tip tested, it will be found to be quite flaccid. Furthermore, measurement shows that the root is now from 0.5–1.0 mm. shorter than before the current was passed. An exposure of a longer period than two or three minutes results in the root becoming more or less translucent, and on testing after such longer exposure it is found to have become even more flaccid. In the preliminary experiments roots were treated with varying amperage and for different time periods, then fixed, sectioned, and stained. While the cytological pictures were similar except as to degree of intensity of results, they were not comparable with each other; for example, it was difficult to produce the same result with a 0.4 milliampere current and a 0.5 milliampere current by varying the time.

After much experimentation, in an effort to arrive at somewhat comparable cytological results, had ended in failure, it was found

possible to establish in a fairly definite way the quantity of current and the time required to produce death of the root. This was accomplished by numerous trials using a constant amperage, and varying the time factor until exactly the time exposure required (using that particular amperage) to produce death was determined. This method yielded comparable cytological results. Current and time factors were varied from 0.6 milliampere for fifty-two seconds to 0.05 milliampere for thirty to thirty-five minutes. Longer exposures were also made for as long as two hours at 0.01 milliampere.

In the determination of whether or not a certain exposure to current produced death, roots immediately after treatment were planted in moist sawdust as previously described. Examination was made after a lapse of twenty-four hours and record of condition made. Roots so treated, current intensity of 0.3 milliampere for two and a half minutes or longer, will after twenty-four hours appear a chalky white at extreme tip and exhibit considerable shriveling in the region of rapid elongation. If such a root is tested by being drawn lightly between thumb and forefinger, it offers little resistance, and flattens readily. It is quite evident that the entire root tip of 1.5 cm. is dead. If subjected for a less period than two and a half minutes to this current intensity, a majority of the roots will show the shriveling in the region of rapid elongation and above, but the first 4–5 mm. of the root will be more or less translucent, quite different in appearance from the chalky white previously described, and quite firm to the touch. It is evident in these cases that such roots are dead above the first 4–5 mm. of the tip, and that the cells are still in a living condition in the extreme tip. This conclusion is further strengthened by the fact that in a great many cases these roots will show curvatures at the tip. These curvatures take no specific direction with reference to how the current is applied. The seedlings were always set into the apparatus with the cotyledons toward the front, yet the curvatures appeared in every plane, and varied from a slight crook to a right angle curve, or in some cases even a bending back on the main axis to a U-shape. These curvatures suggest unilateral injury which stained preparations in no case reveal, and indicate a problem of great interest upon which it is planned to do further work.

The criteria by which a root was judged to be dead, living, or partly dead are the following. When a root after twenty-four hours was distinctly shriveled and drying in the upper region, that

TABLE I

CURRENT 0.6 MILLIAMPERE, 120 VOLTS; CRITICAL TIME 52 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	5	↑	15	+0.5	4	5	0
2	5	↑	30	+0.2	3	5	0
3	5	↑	40	−0.4	4	4	1
4	5	↓	45	−0.5	3	5	0
6	5	↑	50	−0.4	3	3	2
9	6	↓	50	−1.0	2	2	4
11+15	10	↑	52	−1.1	0	0	10
13+15	10	↓	52	−1.2	1	1	9
7+14	10	↓	55	−1.3	0	0	10
10+12	11	↑	55	−1.1	1	1	10
5	5	↑	60	−0.9	0	0	5
8	5	↑	60	−0.16	0	0	5

TABLE II

CURRENT 0.5 MILLIAMPERE, 110 VOLTS; CRITICAL TIME 65 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	5	↑	20	+1.0	3	5	0
2	5	↑	40	+0.1	3	4	1
4	5	↓	45	−0.2	4	4	1
5	5	↓	50	0.0	4	4	1
6	5	↓	55	−1.1	2	2	3
8	5	↑	55	−0.1	3	4	1
7+13	10	↓	60	−0.3	1	2	8
3, 9, 11	15	↑	60	−0.6	5	4	11
15, 17, 19	16	↑	60	−0.7	3	6	10
10+16	12	↓	65	−0.7	1	1	11
14+18	10	↑	65	−1.1	0	0	10
12	5	↑	70	−0.8	0	0	5
20	5	↓	70	−1.0	0	0	5

portion was regarded as dead. In not a single case in all tests made did this part revive after having reached this stage. A chalky white appearance of the first 4 or 5 mm. of tip, together with the

exhibition of loss of turgidity, by showing little resistance to flattening by gentle pressure (being drawn between thumb and forefinger) was regarded as evidence that this portion of the root was

TABLE III

CURRENT 0.4 MILLIAMPERE, 100 VOLTS; CRITICAL TIME 90 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	6	↑	30	+1.1	4	6	0
2	6	↑	35	+2.0	5	6	0
3	5	↑	40	+1.4	3	5	0
4	8	↑	45	+0.8	5	8	0
5	6	↓	50	+1.5	3	6	0
6	6	↑	55	−0.1	3	5	1
7	6	↓	60	−0.5	2	4	2
8+10	12	↓	65	−0.5	7	9	3
9+11	12	↑	70	−0.5	6	8	4
14	6	↓	70	−0.3	5	5	1
12	6	↑	75	−0.5	4	6	0
13+15	13	↑	80	−0.0	5	8	5
23+25	10	↑	85	−0.6	2	3	7
18+27	10	↓	90	−1.0	0	0	10
16, 24, 26	21	↑	90	−0.7	2	2	19

TABLE IV

CURRENT 0.3 MILLIAMPERE, 90 VOLTS; CRITICAL TIME 2 MINUTES, 30 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	6	↑	50	+1.5	3	6	0
2	6	↓	120	−0.5	2	4	2
3	6	↑	120	−0.3	4	5	1
4+9	16	↑	135	−0.5	0	6	10
6	7	↓	135	−0.7	2	3	4
12	5	↑	140	−0.6	1	1	4
5, 7, 13	28	↑	150	−0.7	0	0	28
10	10	↓	150	−1.0	0	0	10
8+11	18	↑	165	−0.9	0	0	18

dead. When, however, the appearance of the extreme tip was watery instead of chalky white and exhibited distinct turgidity, it was regarded as evidence that the cells were in a living condition. Associated with the latter condition was an increase in length and

the curvatures mentioned. It is entirely possible for the upper portion of the root (the region of rapid elongation and above) to be dead and the tip to continue growth for a short time; in fact, many such cases were found. It is well understood that it is difficult to

TABLE V

CURRENT 0.2 MILLIAMPERE, 70 VOLTS; CRITICAL TIME 4 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
11	5	↓	220	−0.1	1	5	0
3+7	10	↑	225	−0.6	0	4	6
9+12	10	↑	230	−0.6	2	4	6
1+8	21	↓	240	−0.4	0	3	18
4+10	15	↑	240	−0.7	0	0	15
2	8	↑	270	−0.9	0	0	8
5	5	↓	270	−0.4	0	0	5
6	5	↑	300	−0.6	0	0	5

TABLE VI

CURRENT 0.15 MILLIAMPERE, 50 VOLTS; CRITICAL TIME 6 MINUTES, 15 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1+12	10	↑	300	−0.4	1	7	3
2, 13, 16	15	↓	360	−0.8	4	6	9
4, 8, 18	16	↑	360	−0.1	4	15	1
19+27	8	↑	370	−0.4	1	2	6
26	5	↓	375	−0.2	0	0	5
14, 17, 21	15	↑	375	−0.7	1	1	14
22, 25	10	↑	375	−0.7	0	0	10
5	5	↑	390	−0.5	0	0	5
10, 15, 23	15	↑	390	−0.5	0	0	15
3, 6, 7	15	↑	405	−0.5	0	0	15
9+11	11	↓	405	−0.6	0	0	11

set up a criterion of measurement as to when death takes place; in fact, death has been called by some physiologists "a reversible process." The writer believes, however, that these criteria are sufficiently definite as used, and that they are scientifically sound.

In determining the time factor, the current was kept at constant amperage by sliding resistances for a definite time period, the time being measured by a stop watch. The usual practice was to treat seedlings in succession in the same manner (as to time and current),

TABLE VII

CURRENT 0.1 MILLIAMPERE, 40 VOLTS; CRITICAL TIME 9 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	5	↑↓	420	0.6	5	5	0
2+18	10	↓	480	−0.2	1	6	4
3, 5, 8	11	↑	480	−0.2	0	7	4
4, 21	8	↑	510	−0.7	0	1	7
23, 25	9	↑↓	525	−0.4	1	2	7
6, 9, 24	13	↓	550	−0.5	0	2	11
10, 14, 19, 22	20	↑↓	540	−0.4	0	1	19
16	5	↓	570	−0.7	0	0	5
15+17	9	↑	600	−0.6	0	0	9
26	10	↓	600	−0.7	0	0	10

TABLE VIII

CURRENT 0.05 MILLIAMPERE, 30 VOLTS; CRITICAL TIME PROBABLY BETWEEN 32 AND 35 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in minutes	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	3	↑	15	−0.5	0	3	0
2	1	↑	18	−0.2	0	1	0
3+8	7	↑	25	−0.2	0	3	4
10	5	↑↓	28	−0.1	1	1	4
9, 11, 12	16	↑	30	−0.3	3	3	13
13	6	↑	32	−0.4	1	1	5
6	2	↑	35	−0.5	0	0	2
14	4	↓	35	−0.5	0	0	4
4	2	↓	40	−0.2	0	0	2
7	2	↑↓	40	−0.3	0	0	2
5	2	↓	50	−0.5	0	0	2

such group (usually five), being designated as a "lot" in the tables. Roots were exposed for a time, calculated from preliminary experiments to be below that required to kill, then time of exposure increased for next lot and so on until all were killed. When ninety

per cent or over were killed at any particular combination of current intensity and time, this was regarded as the death point. Death points were determined for current intensities of 0.6 to 0.05 milliamperes as shown in the tables. Direction of flow of current is indicated by arrows. When the current was applied with the positive electrode at the tip, the arrow points upward. Under "average loss or gain" is given the loss or gain in length over measurement taken just previous to exposure. Final measurement was always taken twenty-four hours after exposure. Under the heading "dead above only" is given the number in which the region above the first 4 or 5 mm. was killed. This effect was often noticeable as far back

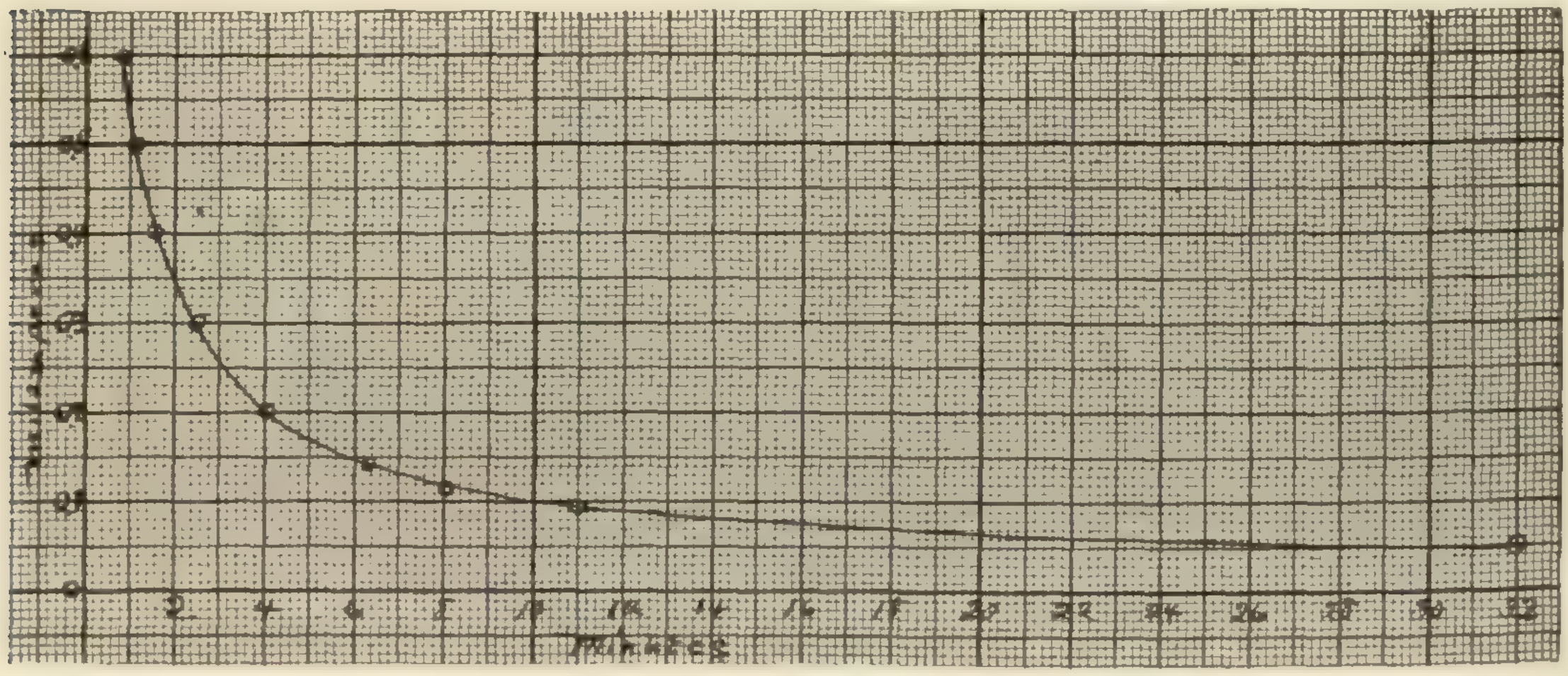

FIG. 3.—Current and amperage required to produce death in roots of 0.7–0.9 sq. mm. cross-sectional area.

as the cotyledons. The last column gives the number in which the entire root was killed. Thus it can be seen from the tables that the critical time at 0.6 milliampere is fifty-two seconds; at 0.5 milliampere sixty-five seconds; at 0.4 milliampere ninety seconds, etc. It should be stated that controls were planted with each lot, and that all showed a decided increase in length, the increase varying from 12–19 mm. at the end of twenty-four hours.

Plotting the points as shown in the tables into a graph gives the death curve (text fig. 3). With current of 0.05 milliampere the death point was determined with great difficulty, due to length of exposure required, which had a tendency to dry out the root. The critical time seems to lie between thirty and thirty-five minutes.

It is not contended that the death curve is sharp and definite. As the tables show, some roots were killed before the maximum was reached. This difference is undoubtedly accounted for by the variations in cross-sectional area of roots used.

Cytological

A rather definite cytological effect is produced in roots exposed to the current just long enough to produce death. Roots exposed for fifty-two seconds at 0.6 milliampere give a very similar picture to roots exposed for two and a half minutes at 0.3 milliampere, or any other point on the death curve. Similarly, roots exposed for any fraction of the time required to produce death at any amperage give a comparable picture with those produced by exposure for the same fraction of time required to produce death at any other amperage. This fact was established only after long experimentation, and until then no comparable cytological results could be obtained with various combinations of time and current. The direction of current through the root had no influence except in direction of migration.

Best results in fixing were obtained by various combinations of Flemming's fluid. Root tips immediately after treatment were placed in the killing fluid. The usual processes incident to the paraffin method were used, and the roots sectioned and stained in saffranin-gentian violet. Satisfactory staining was quite difficult to obtain, and the finer details of the treated protoplasts in most cases were difficult to distinguish.

In a root exposed to the current just long enough to produce death, the cells of the central cylinder back of the root cap show fairly even distribution of cytoplasm, which, however, is coarsely granular compared with the controls. Many cells show a distinct migration of cytoplasm toward the positive electrode. It is in the nuclei, however, that the effect is more noticeable. The nucleolus may have been displaced in either direction, more cells, however, showing displacement toward the positive electrode. A majority of the nucleoli had become elongated in a direction at right angles to the long axis of the root, similar to that shown in fig. 3. The nucleolus frequently is elongated sufficiently to reach

across the nuclear cavity. Other dense material within the nucleus is in every case deposited in a crescent-shaped mass against the nuclear membrane toward the positive electrode. This is shown in some of the cells of figs. 3 and 5. The chromatin appears very coarsely granular. Occasionally a cell is found in which a small amount of this granular material is deposited against the side of the nuclear membrane toward the negative electrode, leaving a clear central space across which lies the much flattened nucleolus.

The cells of the central cylinder about 1 mm. from the cap show shrinkage which is evident at the ends of the cells, but not laterally.

In the cortex of the first millimeter the cells show greater effect than in the central cylinder. The cytoplasm in these cells is in nearly every cell definitely aggregated against the wall toward the positive electrode. The nucleolus no longer lies across the nuclear cavity, but has migrated with the chromatin toward the positive electrode (fig. 8). The nuclear cavity is no longer spherical but egg-shaped, with the smaller end toward the anode. Chromatin and nucleolus are packed into the small end, and seem to have forced distention of the nuclear cavity. Frequently fine granular threads radiate from this mass toward various points in the periphery of the nucleus, as shown in figs. 8 and 11. This bears a striking resemblance to fig. 12, pl. 18, of MOTTIER'S paper (**16**). The nucleolus in most cells at this stage cannot be distinguished as a separate body from the chromatin.

It is in dividing cells that the greatest effect of the current might perhaps be expected, yet such is not the case. Migration does not take place at all in a cell in the process of nuclear division in either cytoplasm or chromosomes. Staining of division figures is poor, and in most cases presents a blurred picture in which the chromatin has the appearance of having melted together. Cells with nuclear division are shown in figs. 2 and 3. This blurred condition is characteristic of all division figures, no matter at what stage. All parts of such a cell usually stain a deep red with saffranin. The absence of migration should be expected in such cells from results found by KITE (**13**) and CHAMBERS (**4**), who both found the protoplast during division in a very viscous state, in the form of a gel.

KITE states that when such a protoplast was cut into, the pieces retained their shape definitely and behaved distinctly as a gel.

In the region of rapid elongation in both cortex and central cylinder, 3–5 mm. from the cap, the cytological picture reveals little effect compared with the extreme tip. The cytoplasm shows no migration whatever, although it is more coarsely granular than that of the controls. The nucleolus retains its form and position, while the chromatin is aggregated in a crescent-shaped mass against the nuclear membrane toward the positive electrode in a majority of cells, in a few toward the negative electrode. In some cases, however, the entire nuclear material is displaced within the cavity (fig. 6), and in a few cases even the entire nucleus lies in a dense mass against the wall toward the positive electrode, a phenomenon not met with at this exposure in cells nearer the cap. Shrinkage is quite evident in the region of rapid elongation.

The root cap rarely shows any displacement of either cytoplasm or nucleus, doubtless largely due to the fact that the moist kaolin of the electrode is a better conductor than the root, and so very little current passes through the cap.

The first noticeable cytological effect of the current is produced on exposure of approximately one-tenth of the time required to produce death, and the first visible reactions occur in the cortical region about 1 mm. above the cap. Such cells show large vacuoles in the end toward the negative electrode, and the cytoplasm appears slightly more granular than in the controls. The chromatin at this stage is beginning to migrate toward the positive electrode. The nucleolus lies in its normal position, but soon after one-tenth of time exposure begins to show the flattening previously described. At this stage the region above the first millimeter shows nothing abnormal, neither do the cells immediately behind the central portion of the root cap. The effect of the current is progressive and the results are cumulative. At one-half of the time for death point, the nucleolus flattens and the cytoplasm definitely begins to migrate. With an exposure of three-fourths of the time for death the nucleolus and chromatin have migrated toward the positive electrode, the cytoplasm being very coarsely granular and exhibiting a greater amount of migration than in the previous

stage. The picture at death point has already been described in detail.

If the current is continued for a longer period than is necessary to produce death, all protoplasmic contents, especially the cortical cells of first 3–4 mm. of tip, are aggregated in a dense mass against the cell wall toward the positive electrode, as shown in pl. II, also partially shown in figs. 9 and 10.

Discussion

Within thirty seconds after the current is applied, the resistance in the circuit falls considerably. The resistance, however, was always kept constant by means of the sliding rheostats. Coincident with the drop in resistance, the tiny droplets of liquid appeared on the surface of the root in the region of rapid elongation and above. This suggests increased permeability of the protoplasts and of the cell walls to liquids of the cell sap, and further suggests increased freedom of movement of particles in the sap or cytoplasm or both. The consequent loss of turgidity and shortening of the root substantiate this view.

It is most interesting to find that the greatest visible effect of the current is not in the region of most rapidly dividing cells, but slightly farther from the tip. This suggests that the protoplasm of these cells exists in a much more viscous state than in cells farther from the tip. The lack or presence of free ions would influence conduction of current. It is possible that free ions exist in increasingly greater number with the absorption of water from the primordial meristem to the region of greatest elongation. If this assumption is true, we would expect least effect of current in the primordial meristem, where the cytoplasm would be viscous and behave as a gel, and a greater effect where the cytoplasm became more nearly semi-fluid, and least effect where the free ions of the cell sap conducted the current almost altogether. This assumption agrees with the facts, for the least (or no) migration occurs in the cells with large vacuoles.

In no preparations made could any basis be found for HARDY'S (9) statement that the cytoplasm migrates to the wall, loses its original charge, gains one of opposite sign, and then migrates toward

the opposite end of the cell. This statement, however, may apply to the chromatin. No theory is advanced as to why neighboring cells behave variously in this regard. The relative hydrogen ion concentration of the different parts of the cell no doubt plays a large part with reference to its reaction to the current. The assumption is generally made that cytoplasm is weakly alkaline. This conception is probably based on the reaction of the cell sap; nevertheless we have evidence that the migration of the protein constituents in the cells is toward the positive electrode. Likewise it is in accord with HARDY's (9) results in treatment of a derived albumen, first with acid, then alkali, and a consequent change in direction of migration as previously stated. That the chromatin under some conditions bears a positive charge seems to be suggested by my own experimental evidence. That the migration of protoplasmic particles, as influenced by the current, is due to the particular electrical charge of the constituent colloidal particles also seems probable, and would suggest that the cytoplasm carries (in the roots of the plants used in this study) always a negative charge.

Summary

1. A method of subjecting roots in moist air to the direct electric current has been devised which makes it possible to control and accurately measure the current actually flowing through the root. There is no evidence that under such conditions roots behave differently from those in soil or liquid if subjected to the same current intensity.

2. Combinations of current intensity and time factors have been determined for producing death of the cells of roots, and the death curve plotted.

3. Cytological preparations of treated roots show a migration of cell contents (with few exceptions) toward the positive electrode.

4. The migratory effect (transfer of material) is not the same for all regions of the root. With one-tenth of time of death current, the cells immediately back of the root cap show little effect, those a little older (1 mm. back) greatest effect, and the cells with large vacuoles no or little effect as to cytoplasm.

5. It is suggested that, with addition of water, more free ions may occur to conduct the current; that the protoplasts of the primordial meristem are in a state of gel.

6. It is further suggested that the difference in true acidity, H-ion concentration of various protoplasts, may account for occasional different behavior of adjacent cells.

7. The theory of electrophoresis probably accounts for the migration phenomena, assuming that the constituent colloidal particles of protoplasm bear an electric charge.

8. Assuming an electric charge carried by such particles, it would follow that the particles of the cytoplasm of the cells of the roots of the Canada Field Pea bear a negative charge, and that the chromatin particles, in some cases only, may bear a positive charge.

The writer takes pleasure in extending thanks to Dr. W. G. MARQUETTE and Professor R. A. HARPER for kindly suggestions and criticism freely given throughout the progress of the work. The work was done in the laboratories of the Department of Botany, Columbia University, New York City.

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EXPLANATION OF PLATES II, III

PLATE II

Longitudinal sections of root tips: magnification shown by scale; smallest spaces equal to 0.01 mm.; ×110.

A.—Root exposed to current of 0.4 milliampere for one and a half minutes; positive electrode at tip.

B.—Root exposed to current of 0.2 milliampere for four minutes; negative electrode at tip.

C.—Control, not exposed to current.

PLATE III

Cells from various portions of roots exposed to current, and from controls; magnifications for all figures approximately 580 diameters, with exception of fig. 8, about 500 diameters, and fig. 10, 550 diameters; all photomicrographs mounted so as to have positive electrode below.

FIG. 1.—Normal cells (not exposed to current) 8–10 mm. from tip in cortex.

FIG. 2.—Cells about 1 mm. from tip in central cylinder from root exposed to current of 0.5 milliampere for sixty-five seconds; in the lower right hand protoplast dividing.

FIG. 3.—Cells from cortex of root exposed to current of 0.2 milliampere for one minute, this being one-fourth time required to kill root at this amperage.

FIG. 4.—Normal cells from cortex about 3 mm. from tip.

FIG. 5.—Cells of central cylinder about 8 mm. from tip; root exposed to current of 0.6 milliampere for twenty-five seconds.

FIG. 6.—Cells from cortex about 10 mm. from tip; root exposed to very severe treatment (ten minutes at 0.4 milliampere).

FIG. 7.—Normal cells from cortex about 1 mm. from tip.

FIG. 8.—Cells from cortex about 1 mm. from tip of root exposed to current of 0.6 milliampere for forty seconds.

FIG. 9.—Cells from cortex about 4–5 mm. from tip; cytoplasm and nuclei also displaced; current of 0.3 milliampere for two and one-half minutes.

FIG. 10.—Same as fig. 9 but exposed to current only one and one-half minutes.

FIG. 11.—From same region as fig. 8

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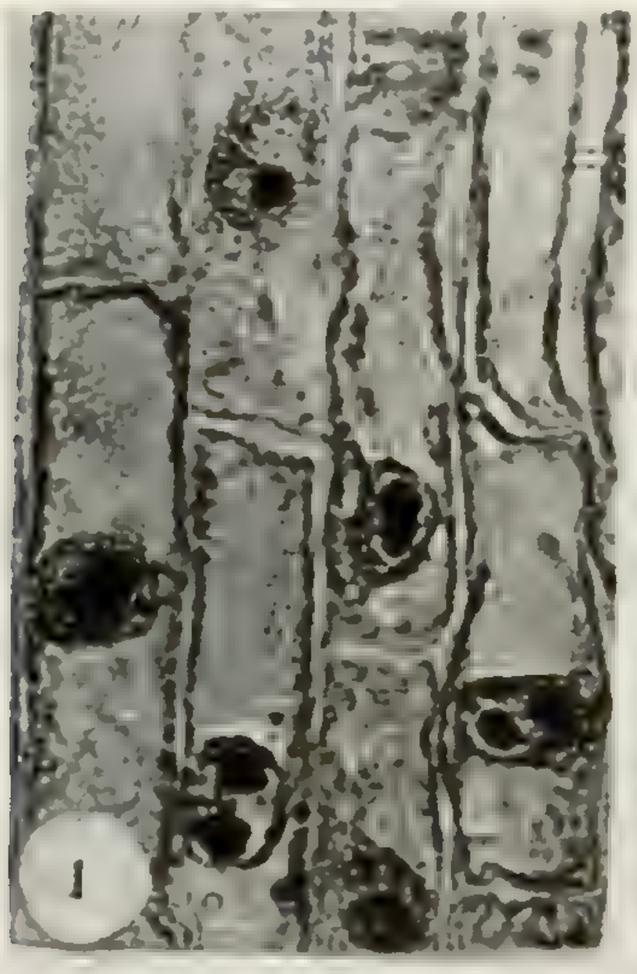

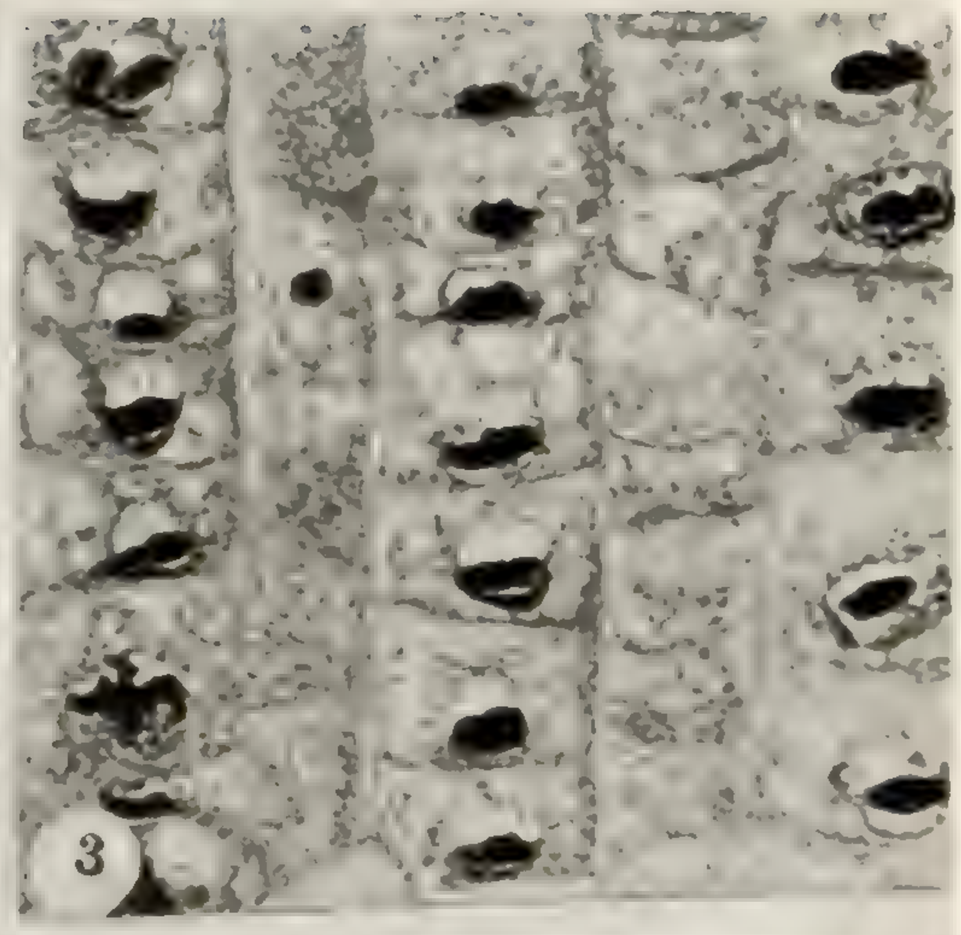

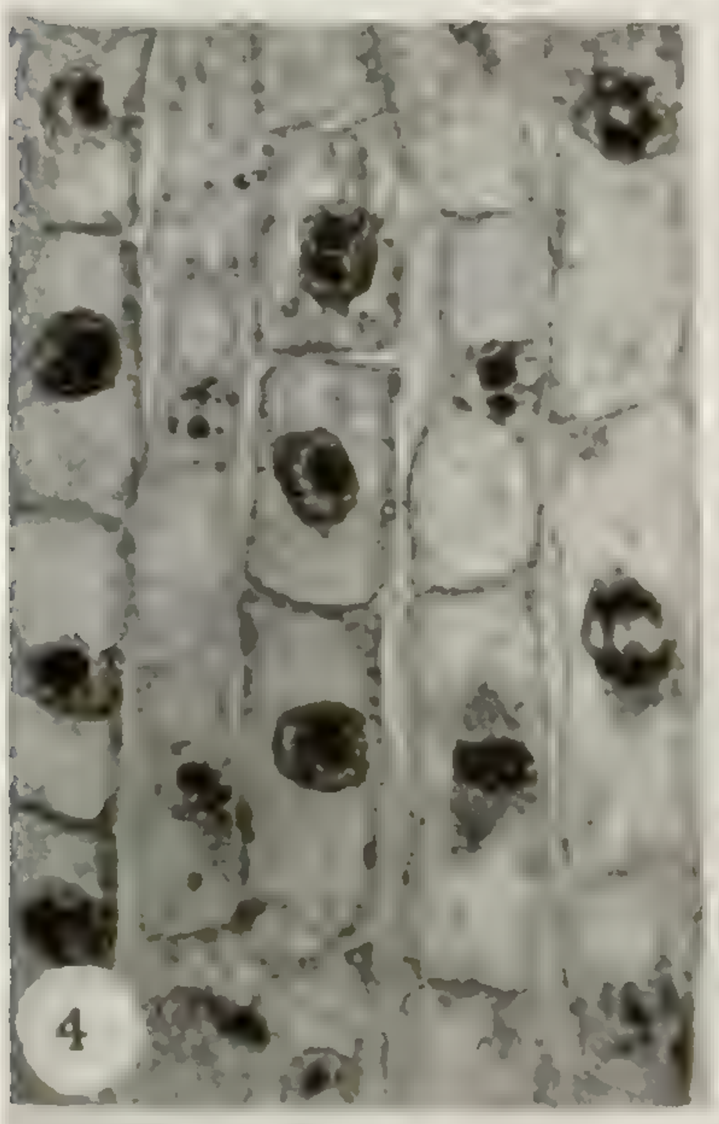

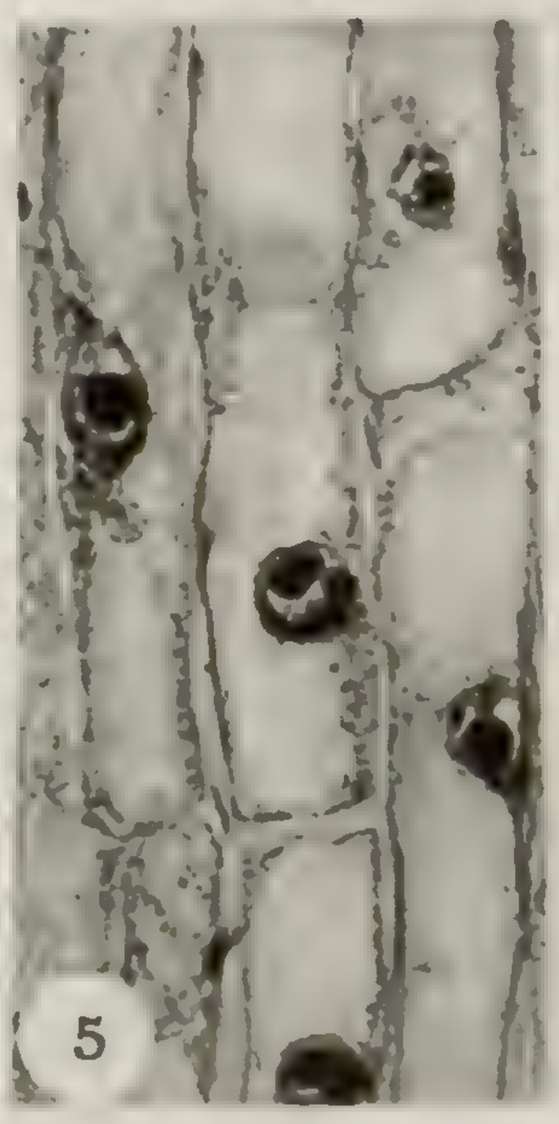

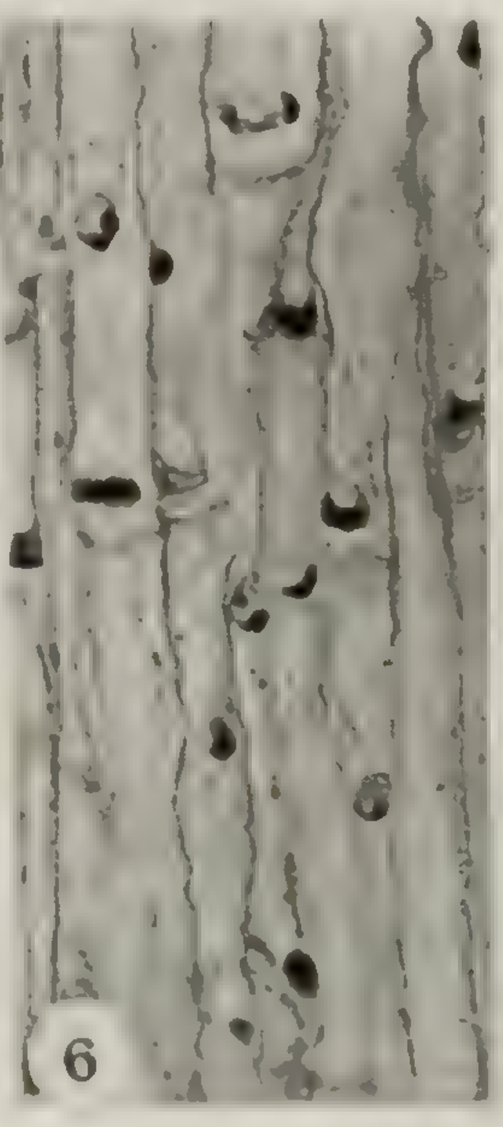

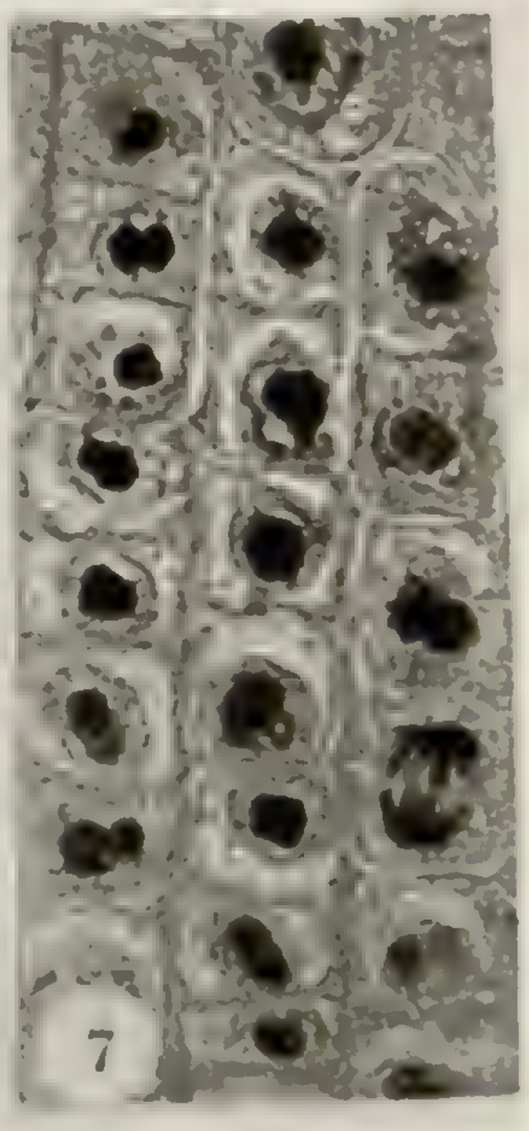

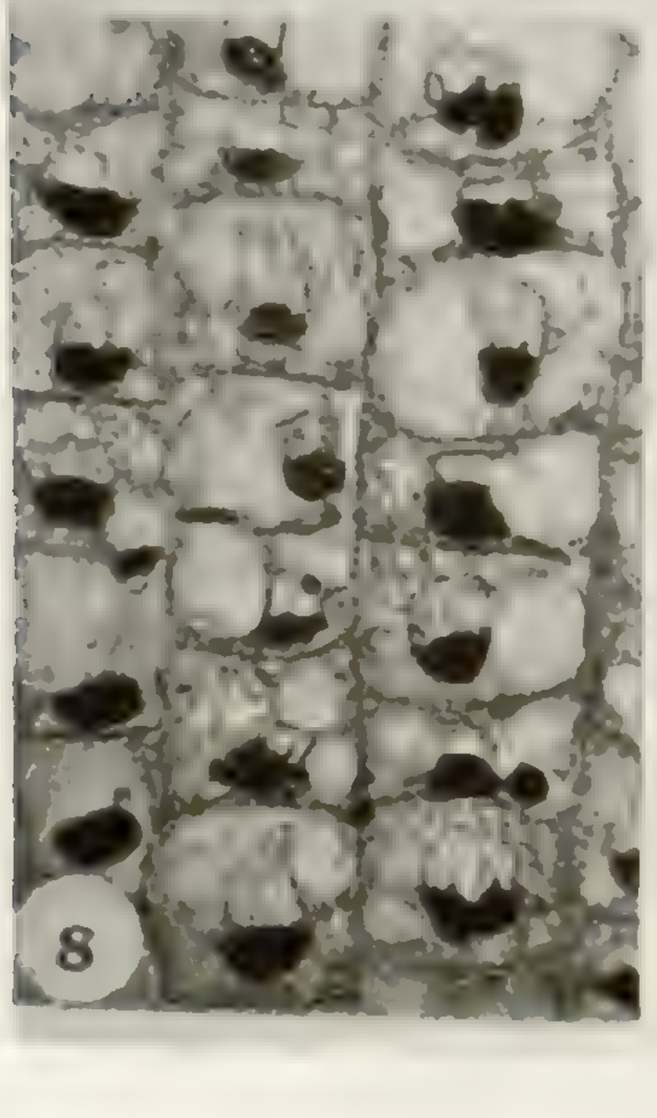

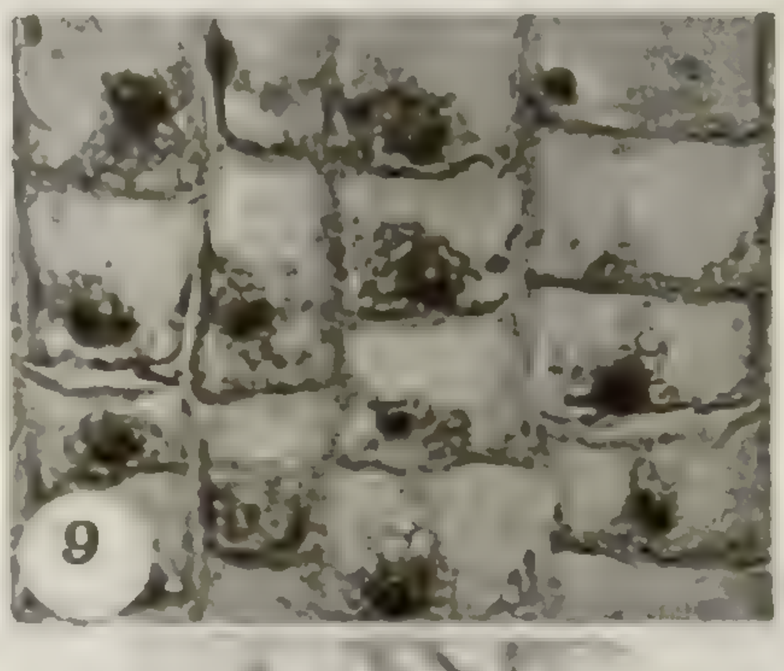

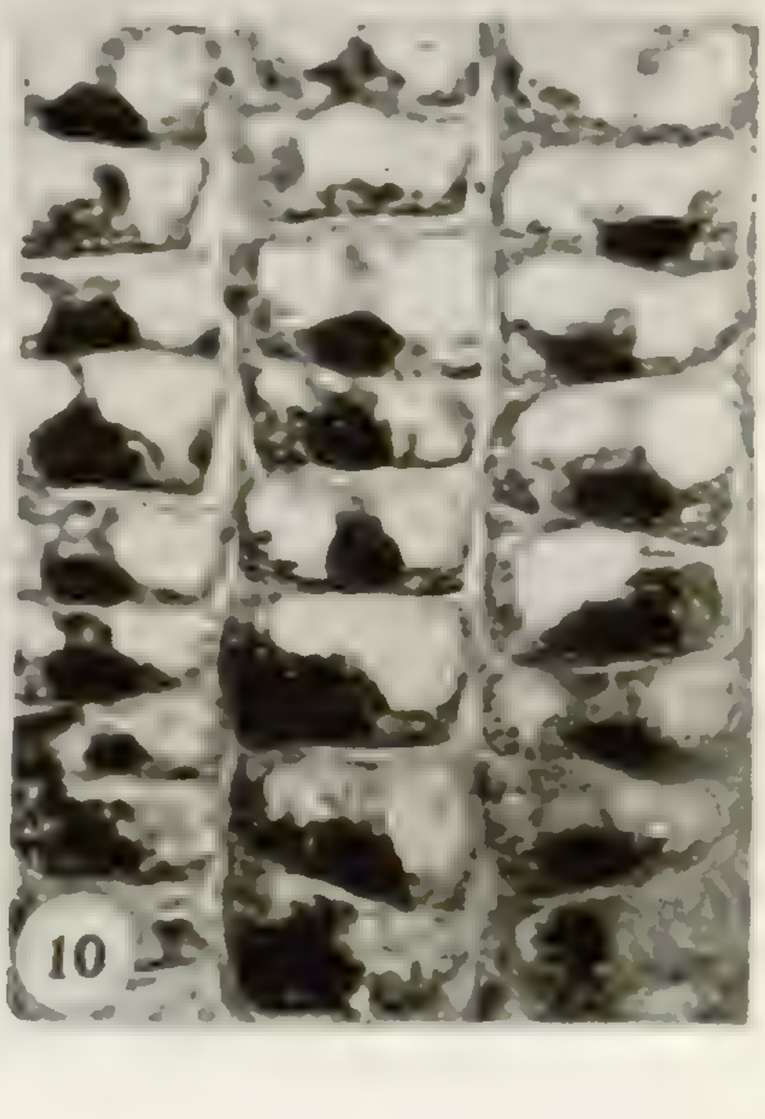

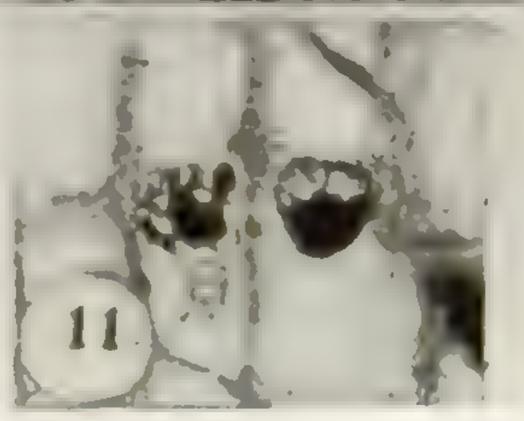

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CHEMISTRY OF AFTER-RIPENING, GERMINATION, AND SEEDLING DEVELOPMENT OF JUNIPER SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 284

DEAN A. PACK

Introduction

In a previous paper the author (17) reported the microchemical and physical changes accompanying the after-ripening, germination, and seedling development of juniper seeds. This work was undertaken with the idea of studying the physiological and chemical changes occurring in the fats during the after-ripening and the seedling development of seeds of *Juniperus virginiana*.

Historical

As early as 1842 DE SAUSSURE (3), while studying the germination of hemp, madia, and rape seeds, discovered two important results that accompany the germination of oily seeds. He concluded that oily seeds during germination absorb a larger volume of oxygen than the volume of carbon dioxide given off, and that the percentage of reserve oil decreases and the percentage of sugar increases during germination. Part of DE SAUSSURE'S work was later confirmed by the investigations of HELLRIEGEL (9) and others.

SACHS (20) in 1859 studied the transformation of oil in many seeds, and concluded that starch was directly derived from oils. PETERS (19) held this same view, but FLEURY (4) denied the constant appearance of starch, and stated that sugar appeared first. The latter investigator was the first to note the probable appearance of organic acids during germination.

MÜNTZ (16) was the first to discover the presence of free fatty acids in germinating oily seeds. While working on rape, poppy, and radish seeds he found that the oil gave rise to free fatty acids. He also noted that this free fatty acid increased several fold during germination. Although the presence of glycerine was not

demonstrated, he concluded that the oil was split up into free fatty acid and glycerine.

GREEN (7), while investigating the reserve products of *Ricinus communis* seeds during germination, discovered the enzyme lipase. He proved that this enzyme was capable of splitting the glycerides of this seed into glycerine and fatty acid. This investigator concluded that glycerine gave rise to sugar, while the fatty acids gave rise to vegetable acids. He also demonstrated the presence of a trypsin-like enzyme, which digested proteids. In a later paper (8) he continued the same investigation and worked especially on the lecithin and sugar content. The lecithin was thought of as being derived from the oils, phosphatic globoids, and proteins. In this paper he discussed the improbability of sugar being formed from glycerine.

In 1895 LECLERC DU SABLON (13) investigated many seeds and finally concluded that saccharose or a nearly related sugar was derived from oils without the glycerine being set free, as in ordinary saponification. MILLER (15), in his studies on the sunflower, records the gradual disappearance of reserve oil and protein material from the cotyledons, with the increase of sugar and protein-free nitrogen in the hypocotyl and roots. In 1912 IVANOW (10) followed the transformation of the oils in seeds during germination. He chose for his work seeds having saturated fatty acids, and others having unsaturated fatty acids. The unsaturated fatty acids were found to be transformed first, and later the saturated fatty acids were used. He ascribed the fall in the iodine number of the fats to the more rapid transformation of the unsaturated fatty acids to carbohydrates, and not to the formation of acids of shorter chains.

KOSSEL (12), as early as 1891, believed lecithin to be present in all protoplasts. STOKLASA (21) states that the phosphatides of rape seeds during five days' germination increased from 0.45 to 5.22 per cent. The dry beet seed with a phosphatide content of 0.45 was found to contain 1.78 per cent after nine days' germination. CZAPEK (2) quotes data from SCHULZE and his school, also others, showing the general distribution and percentage of phosphatides in plant tissues. FRANKFURT (6) in 1894 studied the seeds and

seedlings of the sunflower, and found that glutamin and asparagin increased during germination. PALLADIN (18) in 1896 stated that an increase of the proteins indigestible in gastric juice (nearly proportional to the amount of nucleo-proteins) accompanied the germination of wheat in darkness. ZALIESKI (22) in 1911 reported the general appearance of nucleo-protein in plant tissues. He also found an increase of nucleo-protein with the germination of wheat and corn seeds.

Investigation

CULTURE METHODS

After the seeds had been prepared, as described in an earlier paper (17), they were placed on moist filter paper in Petri dishes and subjected to a temperature of about 5°C. in darkness for after-ripening and germination. Distilled water was added at intervals to keep them moist. The changes occurring had to do with the reserve material already in the seed. The after-ripened seeds and seedlings were kept under these conditions until being prepared for analysis. Analyses were made at three different stages: dry seeds, after-ripened seeds, and late seedling development.

DRY SEEDS.—The hard coats were removed from the dried seeds before preparing them for analysis. In this, as well as in the following stages, the embryo and endosperm (or more exactly, the nucellus, megaspore membrane, and all parts surrounded by these two structures) were the parts analyzed.

AFTER-RIPENED SEEDS.—The seeds were found to require 100 days' storage in a germinator at about 5°C. for after-ripening (or to become ready for immediate germination). The seed at this particular period has already split open the hard coat, and the hypocotyl is breaking through the nucellus. It was at this time that the after-ripened seeds were removed from the hard coats and prepared for the analysis.

DEVELOPED SEEDLINGS.—It required 35 days at 5°C. for the after-ripened seeds to become developed seedlings, which were between 3 and 4 cm. long. The cotyledons were extended and free from the old endosperm, nucellus, megaspore membrane, etc. These latter structures were collected and put with the seedlings

so as to have comparable analytical results. The hard coats were separated and discarded, just as in the collection of the seed material.

Analytical methods and results

The material for analysis was prepared according to Lowenstein (14) and Miller (15), except for slight modifications. The collected seeds and seedlings were thoroughly ground with 95 per cent alcohol in a mortar; then the material was transferred to evaporating dishes and the alcohol evaporated. After thus treating the material three times with 95 per cent and twice with absolute alcohol, it was dried in a vacuum at 75°C. for one hour. The material was then powdered and placed in the desiccator until analyzed. When analyzed the material was in perfect condition, and showed no signs of oxidation.

The method followed in the analysis was outlined by Koch (11). As it was necessary to make both fat and protein analysis on the same sample, the acid precipitation of the lipoid fraction, as earlier described by Koch, could not be used because of possible protein hydrolysis. The lipoids, therefore, were extracted by an 18-hour continuous extraction with hot absolute anhydrous ether. Calcium chloride tubes were used to protect the material from moisture during the extraction. Lipoid or ether soluble material is referred to as F_1. The whole of the lipoids were not dried to get the true weight, because of the danger of oxidizing the unsaturated compounds. This lipoid weight was derived by subtracting the weight of the dry lipoid-free material from the original dry weight. Such a change made it possible to analyze the lipoids at once and avoid oxidation (table II); then the lipoid-free material was extracted with hot 50 per cent alcohol for 12 hours. This 50 per cent alcohol soluble material is indicated as F_2, or extractives. These extractives were dried to constant weight in vacuum, dissolved in hot water, and portions taken for the analysis. The 50 per cent alcohol insoluble material (or F_3) was dried in vacuum, weighed, powdered, and portions taken for analysis (table IV).

Table I gives some general data. The amount of water and solid material found in the air-dry seeds, after-ripened seeds, and seedlings at the time the material was prepared for analysis, is

given as percentage of seed weight with hard coats removed. Total nitrogen is given as percentage of total dry substance and was obtained by the KJELDAHL method. The analysis of chlorophyll

TABLE I

MATERIALS	DRY SEEDS			AFTER-RIPENED SEEDS		SEEDLINGS	
	a	b	c	a	b	a	b
Water	7.19	7.25	7.15	52.64	53.01	88.38	88.54
Solid material	92.81	92.75	92.85	47.36	46.99	11.62	11.46
Chlorophyll as depth of color	.....	.....	.....	Traces	Traces	100.00	100.00
Dry weight of total nitrogen	5.70	.....	.....	5.70	.....	5.72	.....

could not be attempted. The amount of chlorophyll present, however, is given as percentage, and was estimated from the depth of color, considering the chlorophyll content of the seedlings as 100 per cent.

TABLE II

LIPOIDS (F_1)

MATERIALS	DRY SEEDS		AFTER-RIPENED SEEDS		SEEDLINGS	
	a	b	a	b	a	b
Total lipoids as percentage total dry weight	53.60	53.69	43.93	44.01	11.72	11.00
Phosphatides as percentage total dry weight	1.23	1.22	2.80	2.84	2.36	2.37
Acid value as percentage ether extract	1.97	1.90	5.68	5.09	27.75	28.12
Saponification number	174.7	172.3	178.3	180.0	126.0	127.1
Iodine number	133.6	135.0	132.1	131.0	121.4	122.0
Neutral fats as percentage ether extract	95.73	95.82	87.93	88.46	52.07	50.01
Percentage of P in total dry weight	0.03	0.03	0.109	0.110	0.092	0.095
Percentage N as percentage of total dry weight	0.01	.....	0.05	.....	0.05	.....
Percentage of increased weight due to probable O_2 absorption	9.9	.....	11.1	.....	2.1	.....

In tables II, III, and IV the amount of substance found has been given as percentage of the total dry substance unless otherwise stated. Thus in table II the acid value, saponification number, iodine number, and neutral fats were determined for the total lipoid

fraction. The percentage of phosphatide was estimated from the lipoid; P times the factor 25.77. WIJ's iodine solution was used in the determination of the iodine number. No direct nitrogen determination was made on the lipoid fraction. The percentage of nitrogen given was found by subtracting the extractive and protein nitrogen (tables III and IV) from the total nitrogen given in table I. Table II also gives the percentage of oxygen taken up by the lipoid material under artificial oxidation.

TABLE III

EXTRACTIVES (F_2)

PERCENTAGES OF TOTAL DRY WEIGHT	DRY SEEDS			AFTER-RIPENED SEEDS			SEEDLINGS		
	a	b	c	a	b	c	a	b	c
Total extractives	6.68	6.50	....	15.24	15.55	....	34.02	34.59	....
Total nitrogen	0.13	0.12	....	1.04	1.10	....	2.05	2.11	....
Ammonia nitrogen	0.0004	0.0004	....	0.0004	0.0003	....	0.0001	.....	....
Amino acid nitrogen	0.04	0.05	0.04	0.27	0.25	0.30	0.92	0.95	0.93
Reducing sugars after hydrolysis	1.27	1.30	1.25	1.99	1.80	1.88	7.49	7.43	7.55
Direct reducing sugars	Traces	.....	....	0.07	.....	0.05	1.35	1.34	1.37
Reducing sugars after removal of tannins	Traces	.....	....	0.69	.....	....	.....	.....	....
Pentose reaction	0	.....	....	Marked	.....	....	Very marked	.....	....
Unaccounted for material (organic acids)	.....	.....	....	2.7	.....	....	9.7	.....	....

Table III gives the extractives as percentage of total dry substance. The total nitrogen was determined by the BOCK and BENEDICT (1) modification of the FOLIN-FARMER (5) procedure. Ammonia nitrogen was determined by the same procedure after aerating under diminished pressure a large part of the extraction solution made slightly alkaline and collecting the ammonia in dilute HCl. The undistilled material was neutralized with acetic acid, concentrated, and used for van Slyke amino nitrogen determinations. A third portion was used for the sugar determinations. The tannins were removed with pure casein. Both after-ripened seeds and seedlings gave indications of pentose.

The proteins, polysaccharides, etc., are given in table IV as percentages of total dry substance. The protein nitrogen, which is stated as percentage of protein, was determined by the KJELDAHL procedure. Polysaccharides were determined by the MONSON-WALKER and BERTRAND method. This material gave very marked pentose reactions. The cellulose was not determined as such.

TABLE IV

PROTEINS, POLYSACCHARIDES, ETC. (F_3)

PERCENTAGES OF TOTAL DRY WEIGHT	DRY SEEDS		AFTER-RIPENED SEEDS			SEEDLINGS	
	a	b	a	b	c	a	b
Proteins, polysaccharides, etc..	39.73	39.00	40.83	40.43	.....	54.25	54.49
Total proteins ($F_3 \times 6.25$).....	34.21	34.10	28.50	27.95	.....	22.31	22.70
Total hydrolyzable sugars.....	0.0	0.0	0.2	0.23	0.19	14.91	14.79
Indications of pentoses.....	0.0	0.0	Marked	.....	.....	Very marked	.....

Discussion

These results force upon one's attention the great constructive changes as compared with the destructive changes. The major fractions seem to be well accounted for. Such a condition can only be understood when one considers that these results deal with a seed that requires long continued after-ripening and germination at a very low temperature. Although the seed material was kept at a temperature of about 5°C., the constructive metabolism went on at a rapid rate. The digestion of storage fats and proteins was accompanied by the synthesis of many formative and metabolic compounds. The rate and extent to which these changes were carried on even at 5°C. prove the power and efficiency of enzyme action. This low temperature, by retarding respiration, reduced the combustion of materials to a minimum, and thereby favored the accumulation of formative materials in the cells. This accumulation of cell building and cell active materials, together with the culmination of enzymes, probably leads to the after-ripening of dormant organs.

The lipoids decreased 9.7 per cent during after-ripening, and 32 per cent during the seedling development. It will be seen that

the neutral fats sustained this loss. The respiration occurring during the after-ripening period amounted to only 2 per cent, while during the seedling period it amounted to about 5 per cent of the total dry weight. This small amount of material used by the respiration compared with the large amounts of formative, storage, and structural material, high in oxygen content, made from the apparently small amounts of fats, low in oxygen content, will easily account for the low respiratory quotient reported in an earlier paper (17) for these seeds during germination.

The phosphatides more than doubled during the after-ripening process. Glycerine and fatty acids were supplied by the hydrolysis of fats, while phosphoric acid and nitrogen-containing complex were probably derived from inorganic phosphorus and the protein hydrolysis which accompanied the after-ripening. A slight decrease in the amount of phosphatides occurred during seedling development. This decrease could represent the phosphoric acid necessary for the formation of the nucleic acid, which was constructed at this period.

The acid value of the ether extract increased during both after-ripening and seedling development. The iodine number decreased, while the saponification increased slightly without a marked appearance of carbohydrates. Such a condition would probably accompany the breaking up of long carbon chains into shorter chained compounds. The increased fall in the iodine number during the seedling development was due perhaps to the more rapid transformation of unsaturated fatty acids to carbohydrates (10). This carbohydrate accumulation during seedling development amounted to 20 per cent (tables III and IV). The saponification number reached a minimum value for the seedlings, indicating a large percentage of long chained fatty acids. This is accounted for by the large percentage of phosphatides in the seedling lipoids. It appears that these values change materially in the same tissues with different stages of development.

Dry seed and after-ripened seed lipoids were made to take up respectively 9.9 and 11.1 per cent of increased weight due to probable oxygen absorbed by artificial oxidation. There was a slight increase in the reducing power of the lipoids during after-ripening.

Under the same conditions the seedling lipoid material increased in weight only 3.1 per cent due to oxygen absorption.

Of considerable interest is the increase in extractives with after-ripening and seedling development. This is represented by increasing amounts of amino acid nitrogen, and other forms which probably represent amides, peptides, nucleic acid derivatives, alcohols, etc. It also represents increased amounts of various sugars, and very probably organic acids. The ammonia nitrogen value did not change during after-ripening, although it did decrease during the seedling development. This decreasing amount corresponds to the amount of nitrogen required during this same period to build the chlorophyll. As ammonia plays such an important part in the synthesis of proteins (amino acids), however, it is probable that this decrease is of no significance and that the amounts fluctuate. In connection with this it is evident that some proteins, having carbohydrate groups, were rebuilt during the after-ripening and especially the seedling development. Amino nitrogen, van Slyke method, increased about sevenfold during the after-ripening period, and over threefold again during the seedling period (table III). The Formol titration on similar lots of seeds showed a like increase of amino acids during the after-ripening period. The ratio of the amino nitrogen to the total nitrogen of F_2 is as follows: dry seeds one-third, after-ripened seeds one-third, and seedlings one-half. This could mean the formation of shorter-chained amino compounds or the further digestion of peptides, proteoses, or peptones. The increasing amount of non-amino nitrogen during after-ripening and seedling development shows the accumulation of other nitrogenous compounds. This is very probably represented by nucleic acid, peptides, peptones, amides, and other extractives.

Although the sugar formation was very meager during the after-ripening period, it reaches noticeable proportions during the germination and seedling development. Table III shows a 0.5 per cent increase of reducing sugars, after hydrolysis, for the after-ripened seeds. This included a few hundredths per cent of direct reducing sugar. It is evident that nearly all of the reducing sugar of the dry and after-ripened seeds is tied up with the tannins.

The dry seeds gave no pentose reactions, while the after-ripened seeds and seedlings gave marked reactions. During the seedling development the percentage of sugars increased manyfold.

Comparing the amount of total extractives and the sum of the analyzed fractions, it will be seen that there is considerable material unaccounted for. After adding an average percentage for ash, however, the 6.68 per cent of extractives for the dry seeds is nearly all accounted for. After adding the same amount for ash in the after-ripened seeds and the seedlings, there remain respectively 3 and 10 per cent of the extractives unaccounted for. It is evident that this material is not proteins or, much less, decomposition products of the same. Such an explanation would require a protein factor of ten or more. It could not be due to an increase of ash because the seeds were kept in distilled water cultures. The sugars by no means account for this unknown material, and a possible explanation is the presence of organic acids. A review of the analytical results of tables II, III, and IV also shows that there is no other way to account completely for the disappearance of so much fat. It is evident, therefore, that at least part of the fatty acids were oxidized to other organic acids. In the course of the analysis (when F_2 was neutralized for ammonia distillation) it was found that the extractives for dry after-ripened seeds and seedlings all gave an acid reaction. No acid had been used thus far in the analysis, and this acidity was evidently due to acids in the tissues. The extractives of the dry seeds were distinctly acid, while the extractives of the after-ripened seeds and seedlings were very acid. It was also noted that more N/10 NaOH was used to neutralize the seedlings than either the dry seeds or after-ripened seeds. These, with previous results, point to the accumulation of organic acids.

Table IV shows an increase of the protein polysaccharides fraction during after-ripening and germination. There was a decrease in the proteins with an increase of starch. During after-ripening, however, there was a 6 per cent decrease of proteins with only a 0.2 per cent increase of starch. Of course much of this protein material appears in F_2 as amino acids and other nitrogenous compounds. Moreover, some proteins after hydrolysis and deami-

nation very probably gave rise to sugars and acids or were respired. The pentose reactions indicate the rebuilding of proteins with carbohydrate groups. During the germination and seedling development the proteins were hydrolyzed to give rise to the amino acids and nitrogenous compounds of F_2, with the formation of some carbohydrates. From the amount of starch (table IV) and sugars (table III) appearing in the seedlings and the carbohydrates required for cellulose structure, it is evident that not only the proteins but still more the fats contribute to the formation of these materials. From the constant quantity of nitrogen in the analysis and the fact that no nitrogen compounds were added, it is evident that the chlorophyll nitrogen was derived from other nitrogenous compounds.

Summary

A review of these results, together with the changes reported in the previous paper (17), give an idea of the many changes accompanying the after-ripening of dormant organs. These changes are represented by the accumulation of cell building materials: acids, phosphatides, active reducing substances, soluble sugars, pentoses, amino acids, soluble proteins, and other nitrogenous compounds; the accumulation of enzymes; the dispersion of materials; and the transformation of storage materials. This rapid accumulation of simple plastic cell materials coupled with minimum respiration and combustion of materials probably forces the dormant organs to activity. One thus sees the awakened active organ as a very unstable structure made up of many unstable compounds. If these changes are not the basis of the after-ripening process, they are found to accompany the after-ripening process.

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LEAF-TISSUE PRODUCTION AND WATER CONTENT IN A MUTANT RACE OF PHASEOLUS VULGARIS

J. ARTHUR HARRIS

Introductory

In a preceding paper[1] it was shown that the survival of the bean plant is in a measurable degree dependent upon the morphological characteristics of the seedling. In 1915 a series of investigations was undertaken to determine, if possible, something of the proximate causes of the differential death rate. It was also hoped that some light would thereby be thrown upon the proximate causes underlying the occurrence of teratological variations in the seedlings of *Phaseolus*. In undertaking this work the assumption seemed justified that if innate physiological conditions which might affect growth be associated with morphological variations, some influence of these factors should be recorded in the size or other characteristics which result from the relatively enormous expansion which the organs of the embryo undergo in the course of germination and the establishment of the seedling.

A first study[2] demonstrated that teratological seedlings in general show a lower capacity for the development of primordial leaf tissue than do normal seedlings grown under as nearly as possible identical conditions. The data then available indicated that a reduction of the volume of primordial leaf tissue is associated with abnormalities of all the abnormal types studied, but that the type of variation influences in some degree the amount of reduction. In these first experiments the conclusions were based on primordial leaves only. The use of such leaves has the obvious disadvantage that they are formed in the seed, and undergo merely an enormous expansion (and possibly a little differentiation) in the germination

[1] HARRIS, J. ARTHUR, A simple demonstration of the action of natural selection. Science, N.S. **36**:713–715. 1912.

[2] ———, Studies on the correlation of morphological and physiological characters: The development of the primordial leaves in teratological bean seedlings. Genetics **1**:185–196. 1916.

of the seed and the development of the plantlet to the stage at which measurements were made. Since the development of the primordial leaves during the germination and establishment of the seedling is relatively great, it seemed quite legitimate to use the weight of green tissue produced by these leaves as a measure of the physiological capacity of seedlings of various types. The fact that these leaves are differentiated in the seed, however, constitutes a valid objection against their use as a sole measure of the physiological capacity of the seedling. For such purposes a constant based upon some organ developed later seemed desirable.

In a second study,[3] therefore, the tissue weight determinations were extended to the trifoliate leaves of the third node, as well as to the primordial leaves of the second node. This leaf was used because groups of plants of more uniform development can be selected at the time of maturity of this leaf, than at any later stage, and because the first compound leaf reaches a degree of maturity sufficient for the purpose of the present study before the primordial leaves are too old to be used. It is possible, therefore, to check results by determinations made on organs differentiated both in age and in structure. In the first investigation the green weight of the leaf tissue served as the fundamental measurement. In addition to this character certain measurements on the sap properties were also made. In the study of the saps some difficulties were encountered, and it seemed desirable to discontinue that phase of the work temporarily and to carry out determinations of dry weight and water content instead. The present study, therefore, has to do only with the green weight, the dry weight, and the percentage of dry matter.

Recent investigations fell into two phases. The first was an endeavor to determine to what extent seedlings which are morphologically aberrant in the race to which they belong also differ from the normal seedling of the race in their physiological characters, in so far as these can be measured by the capacity for the production of tissue. In the second the investigation was extended from intra-racial to inter-racial comparisons, to ascertain if possible to what

[3] HARRIS, J. ARTHUR, Further studies on the interrelationship of morphological and physiological characters in seedlings of *Phaseolus*. Brooklyn Bot. Gard. Mem. 1:167–174. 1918.

extent a highly abnormal race differs from the parental strain from which it originated.

Materials and methods

In this paper the characteristics of a fully heritable teratological race are considered. The material was furnished by a tetracotyledonous race, the origin and general characteristics of which have been considered elsewhere.[4] The tissue of plants of the tetracotyledonous race were compared with those of the normal line from which it originated.

Seeds of the two series grown in the same field in 1917 were germinated in flats of sand in 1919. Four lots of fifty seeds each, two of the tetracotyledonous plants and two of the normal ancestral line, were germinated in alternate positions in the same flat. Conditions, therefore, were as nearly comparable as possible in the germination of the two series. When the seedlings were of the proper size for potting, one seedling of the tetracotyledonous race and one normal control taken from the same flat were transferred to 3-inch pots of soil, where they stood until they were ready for the collection of samples of tissues. Weighings were then made of the primordial leaves in the two cases. Thus, although weight and other characteristics vary from sample to sample because of age and the innumerable slight influences of significance in growth, the aberrant plants and their controls from the very beginning had as nearly as possible identical environment. However much the pairs combined in the same sample may differ among themselves, there seems no possibility of considering that the differentiation here shown to exist between the morphologically typical and the morphologically aberrant individuals is due to any extrinsic cause. In the absence of any knowledge of the amount of variation in the characteristics of the leaves to be investigated, it was impossible to compute in advance the size of the sample which should be taken. Accordingly it was arbitrarily fixed as 100 plants.[5]

[4] HARRIS, J. ARTHUR, A tetracotyledonous race of *Phaseolus vulgaris*. Mem. N.Y. Bot. Gard. **6**:229–244. 1916.

———, De Vriesian mutation in the garden bean *Phaseolus vulgaris*. Nat. Acad. Sci. **2**:317–318. 1916.

[5] Sample 305 contained 96 plants, sample 252 only 81 plants, and sample 259 contained 138 plants.

In work with the variants in normal lines of beans there is no difficulty whatever in distinguishing primordial leaves from those subsequently formed, except occasionally in extreme variations involving stem characters such as would ordinarily be classed as fasciations. In the case of the tetracotyledonous race, however, it is often difficult to distinguish between true primordials (those formed in the seed) and the simple leaves (not compound) formed subsequently. This difficulty was noted in the first paper on the tetracotyledonous race, and two series of countings at different stages of development of the seedling were made to determine to what extent personal equation may affect the constants for number of primordial leaves.

For practical reasons it was not feasible to count the leaves of the tetracotyledonous plants used in these experiments immediately after germination. Countings, therefore, were made just before the samples were taken. The numbers recorded are those of leaves which were regarded as certainly primordial. Those which from their color or texture appeared to be of subsequent development were omitted. In this race filaments are of rather frequent occurrence. These are probably morphologically much reduced leaves, and were also disregarded. Thus the number of primordial leaves is probably on the average slightly under rather than over the true number for the series as a whole. Since we are primarily concerned with a comparison between definite types of seedling classification with respect to number of leaves, this procedure can introduce no sensible error into the results.

Because of some uncertainty as to the leaves which were to be considered primordial and the considerable variation in the stages of development of the compound leaves in the tetracotyledonous plants, it did not seem feasible in the majority of determinations to consider separately the weight of tissue formed by the compound leaves. This, however, has been done indirectly in the case of certain samples based on the plants as a whole.

Data

The data fall in three groups: a series of weighings of primordial leaves of plants unclassified with respect to number of primordial leaves; a series of weighings of primordial leaves of plants classified

with respect to number of primordial leaves; and a series of weighings of total epicotyledonary tissue.

TABLE I

MEAN GREEN WEIGHT PER PLANT AND PER LEAF IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

Sample	Values per plant				Values per leaf			
	Abnormal	Control	Difference	Percentage difference	Abnormal	Control	Difference	Percentage difference
Unclassified								
226.....	0.6991	0.7516	−0.0525	− 6.9	0.1718	0.3758	−0.2040	−54.2
227.....	0.6972	0.7607	−0.0635	− 8.3	0.1680	0.3804	−0.2124	−55.8
228.....	0.6323	0.7568	−0.1245	−16.4	0.1542	0.3784	−0.2242	−59.2
229.....	0.7012	0.6862	+0.0150	+ 2.1	0.1793	0.3431	−0.1638	−47.7
2 leaves								
258.....	0.4520	0.7263	−0.2743	−37.7	0.2260	0.3632	−0.1372	−37.7
305.....	0.5958	0.7994	−0.2036	−25.4	0.2979	0.3997	−0.1018	−25.4
3 leaves								
255.....	0.6313	0.7760	−0.1447	−18.6	0.2104	0.3880	−0.1776	−45.7
273.....	0.5662	0.7189	+0.1527	−21.2	0.1887	0.3595	−0.1708	−47.5
283.....	0.5791	0.7766	−0.1975	−25.4	0.1930	0.3883	−0.1953	−50.2
303.....	0.6577	0.8015	−0.1438	−17.9	0.2192	0.4008	−0.1816	−45.3
319.....	0.6200	0.7817	−0.1617	−20.6	0.2067	0.3909	−0.1842	−47.1
4 leaves								
253.....	0.6703	0.7786	−0.1083	−13.9	0.1676	0.3893	−0.2217	−56.9
264.....	0.5994	0.6671	−0.0677	−10.1	0.1499	0.3336	−0.1837	−55.0
286.....	0.7066	0.8103	−0.1037	−12.7	0.1767	0.4052	−0.2285	−56.3
296.....	0.6556	0.8142	−0.1586	−19.4	0.1639	0.4071	−0.2432	−59.7
298.....	0.7368	0.9028	−0.1660	−18.3	0.1842	0.4514	−0.2672	−59.1
314.....	0.7201	0.7783	−0.0582	− 7.4	0.1800	0.3892	−0.2092	−53.7
320.....	0.7375	0.7529	−0.0154	− 2.0	0.1844	0.3765	−0.1921	−51.0
5 leaves								
254.....	0.6371	0.7639	−0.0998	−13.5	0.1274	0.3685	−0.2411	−65.4
268.....	0.6409	0.6987	−0.0578	− 8.2	0.1282	0.3494	−0.2212	−63.3
287.....	0.7334	0.7867	−0.0533	− 6.7	0.1467	0.3934	−0.2467	−62.7
300.....	0.8032	0.8366	−0.0334	− 3.9	0.1606	0.4183	−0.2577	−61.6
321.....	0.7125	0.7987	−0.0862	−10.7	0.1425	0.3994	−0.2569	−64.3
6 leaves								
256.....	0.7123	0.7268	−0.0145	− 1.9	0.1187	0.3634	−0.2447	−67.3
289.....	0.7351	0.7646	−0.0295	− 3.8	0.1225	0.3823	−0.2598	−67.9
307.....	0.7950	0.8248	−0.0298	− 3.6	0.1325	0.4124	−0.2799	−67.8
7 leaves								
259.....	0.7312	0.7785	−0.0473	− 6.0	0.1045	0.3893	−0.2848	−73.1
Epicotyl								
244.....	1.4388	2.0369	−0.5981	−29.4	.....	.....	.....	.....
246.....	1.5442	1.9133	−0.3691	−19.3	.....	.....	.....	.....
248.....	1.5448	2.0396	−0.4948	−24.3	.....	.....	.....	.....
250.....	1.6325	1.9028	−0.2703	−14.2	.....	.....	.....	.....
252.....	1.0634	1.1512	−0.0878	− 7.6	.....	.....	.....	.....

PLANTS UNCLASSIFIED WITH RESPECT TO NUMBER OF PRIMORDIAL LEAVES.—In preliminary work (samples 226–229) the total weight of primordial leaf tissue in the abnormal seedlings is compared

with the total weight in the control plants irrespective of the number of primordial leaves formed by the individual plants of the tetracotyledonous race. The total number of leaves per plant, however, was determined in these four series.[6] Thus it is possible to give the average weights both per plant and per leaf in the two series. The results show that in three of the four cases the green weight as given in table I of the approximately four primordial leaves of the tetracotyledonous race is lower than that of the two primordial leaves of the dicotyledonous strain. The percentage differences in total weight range from +2.1 to −16.4, with a general average of −7.37. When the comparison is made on the basis of mean weight per leaf, the primordial leaf of the abnormal seedling is found to be on the average 54.22 per cent lighter than the leaf of the normal seedling.

For dry weight, given in table II, all four series show lower average weight in the tetracotyledonous strain. The percentage differences for dry weight of primordial leaves per plant vary from −1.6 to −18.0, with a general average of −10.90. On the basis of mean dry weight per leaf, the weight for tetracotyledonous plants is found to be from 49.6 to 59.9 per cent lower than that of the normal seedling, with a general average percentage difference of −55.92. Thus the results for these four samples clearly indicate that an abnormal race shows the same relationship to the normal parental race as do abnormal individual seedlings to the normal seedlings in the same race.

PLANTS CLASSIFIED WITH RESPECT TO NUMBER OF PRIMORDIAL LEAVES.—Upon the completion of this preliminary comparison it seemed worth while to analyze the relationships more minutely by considering individually the results for seedlings of the tetracotyledonous race with varying numbers of primordial leaves. These results were only attained at the cost of great labor, since it was difficult to secure considerable numbers of seedlings of any given type simultaneously. It was necessary, therefore, to make determinations for abnormal and control plants in small subsamples, and to combine these to form samples of 100 seedlings

[6] The average numbers per plant were as follows in the four samples: 226=4.07, 227=4.15, 228=4.10, and 229=3.91.

each. The results are shown in table I for green weight, table II for dry weight, and in table III for the percentage of dry matter in the primordial leaves. The data show that in the case of both green and dry weight tissue production is invariably higher in the

TABLE II

MEAN DRY WEIGHT PER PLANT AND PER LEAF IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

SAMPLE	VALUES PER PLANT				VALUES PER LEAF			
	Abnormal	Control	Difference	Percentage difference	Abnormal	Control	Difference	Percentage difference
Unclassified								
226.....	0.0529	0.0600	−0.0071	−11.8	0.0130	0.0300	−0.0170	−56.6
227.....	0.0530	0.0604	−0.0074	−12.2	0.0128	0.0302	−0.0174	−57.6
228.....	0.0528	0.0644	−0.0116	−18.0	0.0129	0.0322	−0.0193	−59.9
229.....	0.0539	0.0548	−0.0009	− 1.6	0.0138	0.0274	−0.0136	−49.6
2 leaves								
258.....	0.0355	0.0587	−0.0232	−39.5	0.0178	0.0294	−0.0116	−39.4
305.....	0.0403	0.0542	−0.0139	−25.6	0.0202	0.0271	−0.0069	−25.4
3 leaves								
255.....	0.0492	0.0649	−0.0157	−24.1	0.0164	0.0325	−0.0161	−49.5
273.....	0.0427	0.0562	−0.0135	−24.0	0.0142	0.0281	−0.0139	−49.4
283.....	0.0404	0.0565	−0.0161	−28.4	0.0135	0.0283	−0.0148	−52.2
303.....	0.0404	0.0539	−0.0135	−25.0	0.0135	0.0270	−0.0135	−50.0
319.....	0.0360	0.0488	−0.0128	−26.2	0.0120	0.0244	−0.0124	−50.8
4 leaves								
253.....	0.0517	0.0641	−0.0124	−19.3	0.0129	0.0321	−0.0192	−59.8
264.....	0.0470	0.0532	−0.0062	−11.6	0.0118	0.0266	−0.0148	−55.6
286.....	0.0493	0.0576	−0.0083	−14.4	0.0123	0.0288	−0.0165	−57.2
296.....	0.0409	0.0540	−0.0131	−24.2	0.0102	0.0270	−0.0168	−62.2
298.....	0.0444	0.0568	−0.0124	−21.8	0.0111	0.0284	−0.0173	−60.9
314.....	0.0444	0.0516	−0.0072	−13.9	0.0111	0.0258	−0.0147	−56.9
320.....	0.0434	0.0478	−0.0044	− 9.2	0.0109	0.0239	−0.0130	−54.3
5 leaves								
254.....	0.0488	0.0604	−0.0116	−19.2	0.0098	0.0302	−0.0204	−67.5
268.....	0.0462	0.0537	−0.0075	−13.9	0.0092	0.0269	−0.0177	−65.7
287.....	0.0501	0.0562	−0.0061	−10.8	0.0100	0.0281	−0.0181	−64.4
300.....	0.0496	0.0549	−0.0053	− 9.6	0.0099	0.0275	−0.0176	−64.0
321.....	0.0423	0.0500	−0.0077	−15.4	0.0085	0.0250	−0.0165	−66.0
6 leaves								
256.....	0.0528	0.0558	−0.0030	− 5.3	0.0088	0.0279	−0.0191	−68.4
289.....	0.0520	0.0555	−0.0035	− 6.3	0.0087	0.0277	−0.0190	−68.5
307.....	0.0496	0.0565	−0.0069	−12.2	0.0083	0.0283	−0.0200	−70.6
7 leaves								
259.....	0.0557	0.0614	−0.0057	− 9.2	0.0080	0.0307	−0.0227	−73.9
Epicotyl								
244.....	0.1048	0.1507	−0.0459	.....	.....	.....	.....	.....
246.....	0.1159	0.1443	−0.0284	.....	.....	.....	.....	.....
248.....	0.1127	0.1514	−0.0387	.....	.....	.....	.....	.....
250.....	0.1193	0.1413	−0.0220	.....	.....	.....	.....	.....
252.....	0.0939	0.1045	−0.0106	.....	.....	.....	.....	.....

two primordial leaves of the normal ancestral strain than it is in the two to seven leaves of the tetracotyledonous strain. The percentage

TABLE III

PERCENTAGE DRY SUBSTANCE IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

SAMPLE	PRIMORDIAL LEAVES		
	Abnormal	Control	Difference
Unclassified			
226	7.566	7.982	−0.416
227	7.601	7.940	−0.339
228	8.350	8.509	−0.159
229	7.686	7.986	−0.300
2 leaves			
258	7.853	8.082	−0.229
305	6.765	6.788	−0.023
3 leaves			
255	7.793	8.363	−0.570
273	7.541	7.817	−0.276
283	6.976	7.275	−0.299
303	6.142	6.724	−0.582
319	5.806	6.242	−0.436
4 leaves			
253	7.712	8.232	−0.520
264	7.841	7.974	−0.133
286	6.977	7.108	−0.131
296	6.238	6.632	−0.394
298	6.026	6.291	−0.265
314	6.165	6.629	−0.464
320	5.884	6.348	−0.464
5 leaves			
254	7.659	8.196	−0.537
268	7.208	7.685	−0.477
287	6.831	7.143	−0.312
300	6.175	6.562	−0.387
321	5.936	6.260	−0.324
6 leaves			
256	7.412	7.677	−0.265
389	7.073	7.258	−0.185
307	6.238	6.850	−0.612
7 leaves			
259	7.620	7.892	−0.272
Epicotyl			
244	7.283	7.398	−0.115
246	7.505	7.541	−0.036
248	7.295	7.423	−0.128
250	7.307	7.425	−0.118
252	8.834	9.083	−0.249

values show considerable variation from sample to sample. As might have been expected on a priori grounds, the deficiency of

the weight of primordial leaves in the tetracotyledonous line is greatest when only two leaves are formed.

A comparison of the average percentage differences for abnormal plants with various numbers of leaves gives the following results:

No. of leaves	Green weight	Dry weight
2	−31.55	−32.55
3	−20.74	−25.54
4	−11.97	−16.34
5	− 8.60	−13.78
6	− 3.10	− 7.93

Only one sample is available for seedlings with seven primordial leaves, and it is omitted from the comparison. The results for the other five classes show that:

a) The difference between the total weight of primordial leaf tissue in the abnormal seedling and its normal control decreases as the number of leaves in the abnormal plant increases, but that throughout the entire range of variation of leaf number studied the tetracotyledonous plant produces a smaller total weight of leaf tissue than do normal plants of the line from which it was derived.

b) The differences between tetracotyledonous and dicotyledonous plants are always greater when the comparison is made on the basis of dry weight than when it is made on the basis of green weight.

If the comparison be made on the basis of average weight per leaf the following results are obtained:

No. of leaves	Green weight	Dry weight
2	−31.55	−32.40
3	−47.16	−50.38
4	−55.95	−58.12
5	−63.46	−65.52
6	−67.66	−69.16

The percentage differences in average weight per leaf of course increase as the number of leaves in the abnormal seedlings increases. Again the greater percentage difference when dry weight serves as a basis of comparison is conspicuous. The percentage of dry matter

in the seedlings of this extremely abnormal race is shown in comparison with the normal control plants in table III. The results are self-explanatory. Without exception, in the twenty-three samples representing weighings of 9958 leaves of abnormal and 4668 leaves of normal plants, the percentage of dry matter is lower in the abnormal than in the control series. A study of the averages for the individual groups of seedlings, classified with respect to primordial leaf number, does not suggest a significant difference in the percentage of dry matter in the different classes of seedlings. Probably a far larger series of weighings would be required to bring out such a differentiation if it exists at all.

TOTAL WEIGHT OF EPICOTYLEDONARY TISSUE IN PLANTS OF TERATOLOGICAL AND NORMAL RACES.—In the foregoing discussion comparisons were limited to the weight of primordial leaves. This was done because of the difficulty of securing leaves subsequently formed in comparable stages of development in the normal and teratological seedlings. It seemed desirable to supplement these studies by the determination of the total weight of tissue produced by the two races. The results for a comparison of the total weight of tissue produced above the cotyledonary node by tetracotyledonous plants and their normal control of line 139, are shown under the heading "epicotyl" in the fundamental tables of data.

The constants show that without exception the green weight and dry weight per plant and percentage dry matter are higher in the normal than in the tetracotyledonous plants. The percentage differences range from −7.6 to −29.4 in the case of green weight, and from −10.1 to −30.5 in the case of dry weight. The differences in percentage of dry matter range from −0.036 to −0.249. The average weight of green tissue per plant is 1.4447 for the abnormal and 1.8088 for the control series, or a difference of −0.3640 gm. The average dry weight per plant is 0.1093 for the abnormal and 0.1384 for the normal seedling, or a difference of −0.0291 gm. The average percentage difference for the green weight is −18.96, while for dry weight the difference is −20.30. The percentage of dry material in the abnormal seedling is 7.6448 as compared with 7.7740 in the control, a difference of −0.1292.

Summary

This paper presents the results of an investigation of green weight, dry weight, and of the ratio of green weight to dry weight in primordial leaf tissue in mutant and parental races of *Phaseolus vulgaris*. The data show that when grown under as nearly identical conditions as possible the primordial leaves of the mutant (tetracotyledonous) show a smaller green weight, a smaller dry weight, and a lower ratio of dry weight to green weight than those of the normal (dicotyledonous) parental race. Thus the tetracotyledonous race is distinguished not merely by striking morphological differences, but by physiological differentiation as well. In this respect the results for the heritable mutant race are in agreement with those for variant individuals within the same strain.

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TECHNIQUE IN CONTRASTING MUCORS

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(WITH TWO FIGURES)

For the last two years a more or less intensive study of the sexual reactions between different races of mucors has been conducted. These reactions have been tested by growing the races side by side in culture dishes under suitable environmental conditions and observing the abundance of conjugations which take place between the races thus "contrasted." In making so large a number of contrasts, it has been necessary to develop a special technique in order to minimize the time consumed in the preparation and handling of the cultures, to obtain greater accuracy in making observations, and to avoid the various experimental errors to which one untrained in the work is liable. As has already been shown, the discordant results obtained by different investigators working with the same species of mucors may be explained by a difference in the technique employed. It has seemed desirable, therefore, to give in some detail the methods used before presenting the results of the investigations in a series of papers which it is hoped to publish shortly.

GROSS CULTURES.—As a source of races to be investigated, gross cultures grown in the laboratory are more convenient than natural cultures found out of doors. Certain forms are characteristic of certain types of substrata. Most mycologists are familiar with the flora obtained from dung cultures made by placing bits of dung on filter paper above damp peat moss in a covered crystallizing dish. Brazil nuts have been found a constant source of certain forms such as *Cunninghamella bertholletiae*, *C. echinulata*, *Syncephalastrum*, and certain other species. In making gross cultures of these nuts it was found convenient to use chiefly the shells, since the meats furnish too luxuriant a supply of nutrient, so that *Rhizopus* is likely to overgrow the less vigorous forms in the culture. The nuts were cracked separately and their shells placed in piles

on filter paper above peat moss in shallow galvanized cake tins covered with glass plates. The shells of each nut were kept separate and placed near the edge of the culture dish in order to facilitate their examination with the hand lens. The hands and the nut-cracker were sterilized with alcohol after cracking each nut, in order to prevent an undue amount of infection of one nut from spores which may have been on the one cracked previously. It was the usual practice to reserve a culture dish for the nuts obtained from a single collection, despite the fact that many of these collections were from stores within a radius of thirty-five miles from New York City, and very probably had the same ultimate source.

Another prolific origin of mucors is soil obtained from different sources. It was most conveniently scattered on bread, which furnished a suitable medium for the germination and growth of the spores which the soil might happen to contain. The bread before using was sterilized in the autoclave without pressure for about 5 minutes, a longer time not being used since it might make the bread soggy. If the surface of the bread has become dry, it may be moistened slightly with sterilized water.

STOCK CULTURES.—The races were obtained in pure cultures by making transfers from individual heads in the gross cultures. Since there is possibility of spores from another race being on the head from which the transfer was made, a second pure transfer was regularly made from an individual head in the first test tube, thus avoiding the likelihood of the stock cultures being mixtures of more than a single strain. As a precaution against too rapid drying out, the amount of agar flour in the stock cultures was raised from 2 to 3 per cent, and standard no. 230 nutrient, consisting of 2 per cent each of dextrose and dry malt extract plus 0.1 per cent meat peptone, was used.

INFECTION.—Knowledge of an investigator's methods may enable the impartial critic to judge whether the known sources of experimental error are properly guarded against. To the bacteriologist and the student of fungi in pure cultures, one of the greatest sources of error is infection. When this infection is caused by a form different in appearance from the species under cultivation, the presence of the foreign growth is readily recognized. Infection

is less readily recognized, however, when the invading growth is another race of the same species. *Syncephalastrum* and *Cunninghamella* are forms which bear their spores externally and shake them off at the slightest touch or breath of air. When a dish containing a mature culture of these species is opened, the spores may be seen to rise in clouds. The spores of *Rhizopus*, although inclosed in a sporangium, are readily scattered into the air by the rupture of the brittle sporanguim wall, and in consequence *Rhizopus* also is a common source of infection. That the air may be filled with viable spores of both sexes of *Cunninghamella* has been shown by exposing Petri dish cultures for a short time and growing them at a temperature suitable for zygospore formation. In the laboratory where *Cunninghamella* had been recently grown, it was always possible to find growths of the same species in Petri dishes exposed in this way, and frequently both the sexual races were obtained, as shown by the production of their zygospores. Experience has shown, therefore, that in working with *Cunninghamella* one must observe greater precautions to avoid air-borne infection with other strains of the same species than is necessary when working with many other forms.

Not only are cultures of *Cunninghamella* especially liable to infection with spores of the same species, but the spores of this species which gain access to a mature culture are able to germinate and grow on the aerial mycelium which they infect. With most mucor species, vigorous growth on the nutrient substratum is necessary before zygospore formation is possible. With *Cunninghamella*, however, connection with the nutrient agar does not appear to be necessary in order to allow the mycelia to assist in zygospore formation, since if spores of one sex are planted on an aerial growth of the other sex, zygospores are likely to be produced. This peculiarity of *Cunninghamella* was responsible in the earlier cultures for the appearance of zygospores where they should not be found on the theory of sexual dimorphism, and led to a repetition of the first series of cultures and a revision of the technique.

NUTRIENT MEDIA.—The method of growing and testing races of *Cunninghamella* for sexual reactions was the same as that adopted for other forms, with such slight modifications as the peculiarities of

the individual species demanded. Two per cent agar with the addition of different nutrients was used as a substratum in all cases. The formula varied with the different species tested. No. 230 standard stock nutrient (already described) was used in the tests when possible. Before starting an extensive series of tests of a given species, however, the effects of a number of different nutrients were tested, and the one chosen which was able to support a relatively abundant production of zygospores. For "imperfect hybridization" reactions no better nutrient was found than no. 362, which is a milk whey agar consisting of 2 per cent agar, 1 per cent dextrose, and 2 per cent dry milk whey powder. Some species form zygospores at laboratory temperatures, while others require a higher temperature for sexual reproduction. The species of *Cunninghamella* investigated belong to the latter class, and accordingly tests of this genus have been grown in the incubating oven at temperatures between 24° C. and 28° C.

CULTURE DISHES.—A suitable culture dish is a matter of some importance, especially when large numbers of cultures are handled together. It should be relatively small in order to economize space, and yet should provide sufficient surface of the nutrient medium to support vigorous growth of the two contrasted mycelia. The danger of infection must be reduced to a minimum, and yet the dishes should be such as to be manipulated easily in being filled with nutrient, inoculated with the races to be tested, stored during incubation, and examined under the microscope. Test tubes, although fitted for holding material in stock cultures, require considerable time to be plugged, filled, and sterilized, and moreover cannot easily be handled for observation under the microscope. Petri dishes, while in many ways superior to test tubes, are expensive, do not stack well, and are furnished with a loosely fitting rimmed cover. The Syracuse watch glass with ground rim for pencil labeling has been found to satisfy all the requirements of a safe and convenient culture dish, and has been used almost exclusively in recent years in testing contrasts between different races. They are conveniently handled in stacks of five, four dishes for cultures and an empty one for the top cover. Each dish, except the bottom one, serves thus as a cover for the one

below. A single stack, therefore, can be used in testing each of two races by contrasts with a plus and a minus tester. The stacks of culture dishes are dry sterilized, and when cool filled with sterilized nutrient agar slightly above the melting point. If the agar is too warm, moisture may condense on the covers and later drop on to the surface of the hardened agar. The process of pouring agar into the dishes is carried on in a special culture room to minimize danger of contamination. The cultures are ready for inoculation as soon as the agar has begun to harden. Pouring agar into sterile dishes rather than sterilizing the dishes after they have been filled not only saves considerable time in the process, but avoids the spattering of nutrient on the edges of the dishes, which is likely to ensue when they are autoclaved, and is a ready source of infection. It has not been found necessary to use cleared agar, but the sediment at the bottom of the flask from which the stacks are poured has usually been discarded. This sediment may be conveniently anchored to the bottom by cooling the flask when full in a shallow pan of water.

INOCULATIONS.—Since forty or more individual races in test tubes may be used in inoculating a given series, it is obvious that some precautions are necessary to avoid contamination from spores falling from these tubes, or from the weft of spore material taken from them for inoculation. Exposed Petri cultures have shown that this danger is great if not guarded against. In making inoculations, great care was taken never to expose to the air in the inoculating chamber any spore material in a dry condition. A fairly large piece of rather soft agar was transferred with a flamed platinum needle or spatula to the tube from which it was wished to obtain a transfer. The piece of agar was cautiously pushed against the spore material, and thoroughly mixed with it, care being observed that no dry filaments adhered to the needle or to the mixture of agar and spore material to be used as the inoculum. Moreover, the cotton plug was not removed suddenly from the test tube, since if this is done spores are likely to be shaken into the air, sometimes in visible clouds. After the inoculation the needle was not at once flamed, as the heated inoculum may sputter and scatter bits containing viable spores. The needle, therefore, was left in a tube of about 80 per cent alcohol while the label was

being written on the culture. This alcohol bath helps to sterilize not only the needle but also the base of the needle holder, which cannot readily be flamed, but which may carry spores from the test tube cultures. The alcohol was burned off and the needle flamed before a new inoculation was made. A layer of shot will be found convenient to weight the jar of alcohol and to receive the point of the needle. It is a rule of the laboratory never to lay a test tube down from which a transfer is being made until the label is written on the new culture.

The first race to be tested was inoculated in a streak at the left and at the right respectively of the first and second culture dishes in the stack. Similarly the second race was streaked at the left of the third dish and at the right of the fourth dish. In like manner other stacks were inoculated with the remaining races to be tested, so that finally a series of stacks was secured with two of the dishes streaked with one race and two dishes with another race. They were then ready to be planted with the testers, which are most conveniently a pair of races of opposite sex. In such a case, the plus was streaked on the right of the first and third culture dishes of each stack, and the minus on the left of the second and fourth dishes. Each dish, therefore, contained a tester and a race to be tested, and each stack accordingly completely tested two races. An advantage in choosing a plus and a minus race as the two testers in a series was that they could be planted together as controls for the production of zygospores. They may be grown in duplicate, and a pair of dishes with nutrient but without inoculations may complete the control stack and give evidence of the amount of infection to which the cultures are liable. In inoculating the testers a larger amount of spore material was rolled up on the needle, which sufficed without renewal for the inoculation of 30 or 40 dishes. With practice the process of inoculation could be carried on with relative rapidity. In inoculating a culture with more than a single race, the needle should be kept on its own side, to decrease the likelihood of spores falling upon a part of the substratum reserved for another race.

When "imperfect hybridization" was expected, the tester and the race to be tested were streaked so that the lines of inoculation formed a V instead of running parallel. In consequence, the two

mycelia met sooner in their growth at one end of the line of contact than at the other, and thus offered a greater area at a given time for the observation of sexual reactions which may soon be covered by the later growth of the mycelium. Obviously the angle of the V must be left open, and care taken in planting a tester to avoid touching with the needle the previous line of inoculation. The distance between the points or lines of inoculations may be a matter of some importance, since certain forms fail to produce zygospores in the line near the inoculations, while others produce them only within a distance of a few millimeters from the inoculations.

After inoculation, the stacks are stored in the incubating oven if greater than laboratory temperature is necessary. If the stacks are inverted until the mycelium covers the agar, the danger is avoided of water condensing on the dishes above and falling upon the cultures, with a consequent running together of the recently made inoculations. It may be found desirable to have jars of water on the culture shelves to prevent the nutrient from drying out before they are finally recorded. Before the cultures had matured, they were inspected to see whether any inoculation had failed to grow. With *Cunninghamella* the inspection was more thorough than with most other forms, and consisted in an examination for beginnings of conjugation such as are seen in "imperfect hybridization." Apart from this early inspection on the first or second day after inoculation, and before there is much danger in disseminating spores from the cultures into the air, the stacks were not opened before being sterilized in the autoclave, generally on the seventh or eighth day. The heat of the autoclave melts the agar and tends to glue the filaments to the bottom of the dish above. It was found convenient, therefore, to have the stacks inverted during sterilization, with a pan below to catch the melted agar. Just before cooling the stacks were erected and each dish lifted in succession. Any of the cultures still adhering to their covers after this procedure may be freed by the use of alcohol. Keeping the dishes closed while the spores are likely to be shed reduces the amount of infection of the laboratory air, which is probably impossible to prevent entirely when a species like *Cunninghamella* or *Syncephalastrum* is cultivated in a wholesale manner. Fig. 1 shows a series

of empty stacks in the inoculating room ready for pouring. A tube of alcohol is seen at the right holding the inoculating needles.

EXAMINATION OF CULTURES.—The cultures were examined under the binocular microscope by the transmitted light of a substage electric lamp. In some species the mycelium and spores above the substratum hid any zygospores which might be present, and necessitated manipulating the culture before examination. Wetting with alcohol, pressing down the aerial growth with the finger, and even washing the spores away under a jet of water occasionally

FIG. 1.—Inoculating chamber with stacks of culture dishes ready for pouring

was found necessary. The relative abundance of zygospores when present is shown by the grades *A* to *D*. *A* indicates about the maximum number of zygospores to be expected of the species under the given environmental conditions, while *D* indicates generally less than a dozen zygospores in the whole culture. Absence of zygospores is indicated by *O*. Naturally the zygospores would be expected to form in greatest numbers at the line of contact between the opposing mycelia, and in some species produce a sharply defined dark line. In forms like *Rhizopus* and *Cunninghamella*, in which the zygospores are produced on branching aerial filaments, the zygospores may spread from this median line until ultimately

they are scattered through the whole culture. They should not be least abundant in the center, however, and a very few isolated zygospores at some distance from the line of contact make one suspicious of possible infection and demand a repetition of the culture.

In the study of incompleted sexual reactions such as are found in "imperfect hybridization," greater care must be exercised than in the observation of zygospores, since the cultures have not been sterilized, and infection may take place in an early examination of the dishes and be the cause of the sexual reactions seen when the cultures are looked at later. At first glass plates were used to cover the cultures, to prevent the delicate filaments from drying and collapsing while they were being examined. This entailed cleaning and sterilizing the plates in alcohol and afterwards drying them, and consumed more time than the procedure finally adopted. By this method a stack was placed on a sheet of wet blotting paper and covered with a crystallizing dish lined with moist paper, thus forming a humidor for the cultures. The stage of the binocular microscope was covered by several layers of wet blotting paper perforated to match the opening in the stage for the entrance of light. A collar of moistened blotting paper formed a moist chamber with the wet paper on the stage and allowed the examination of a culture to continue for some time without collapse of the hyphae. The cover was removed from the stack in the humidor formed by the crystallizing dish and placed upside down on the table. The bottom of the first culture dish was swabbed with a pledget of cotton soaked with alcohol before being put in the moist chamber formed on the stage of the binocular, and after being examined was placed upside down on the inverted cover. The second, third, and fourth cultures were treated in a similar manner, except that the bottom of the fourth culture, which had not been in contact with any culture below it, was not treated with alcohol. After the last dish had been removed from the humidor and examined, it formed the last member of an inverted stack, and a new stack was placed in the humidor ready for examination. From time to time it was found necessary to re-wet or renew the blotting paper on the binocular. The paper on the stage is theoretically capable

of transmitting spores to the cultures examined, but when the dishes were treated with alcohol in the manner indicated, it has not proved a source of error. Fig. 2 shows the arrangement of apparatus in an examination of cultures. At the left are two trays with stacks of cultures ready for examination. Next toward the right is a glass plate containing the humidor and the inverted members of a stack already examined. On the stage of the binocular is a culture under examination. The fifth dish of the stack not yet examined is covered by the humidor. At the extreme right is a series of inverted stacks which have already been examined. The

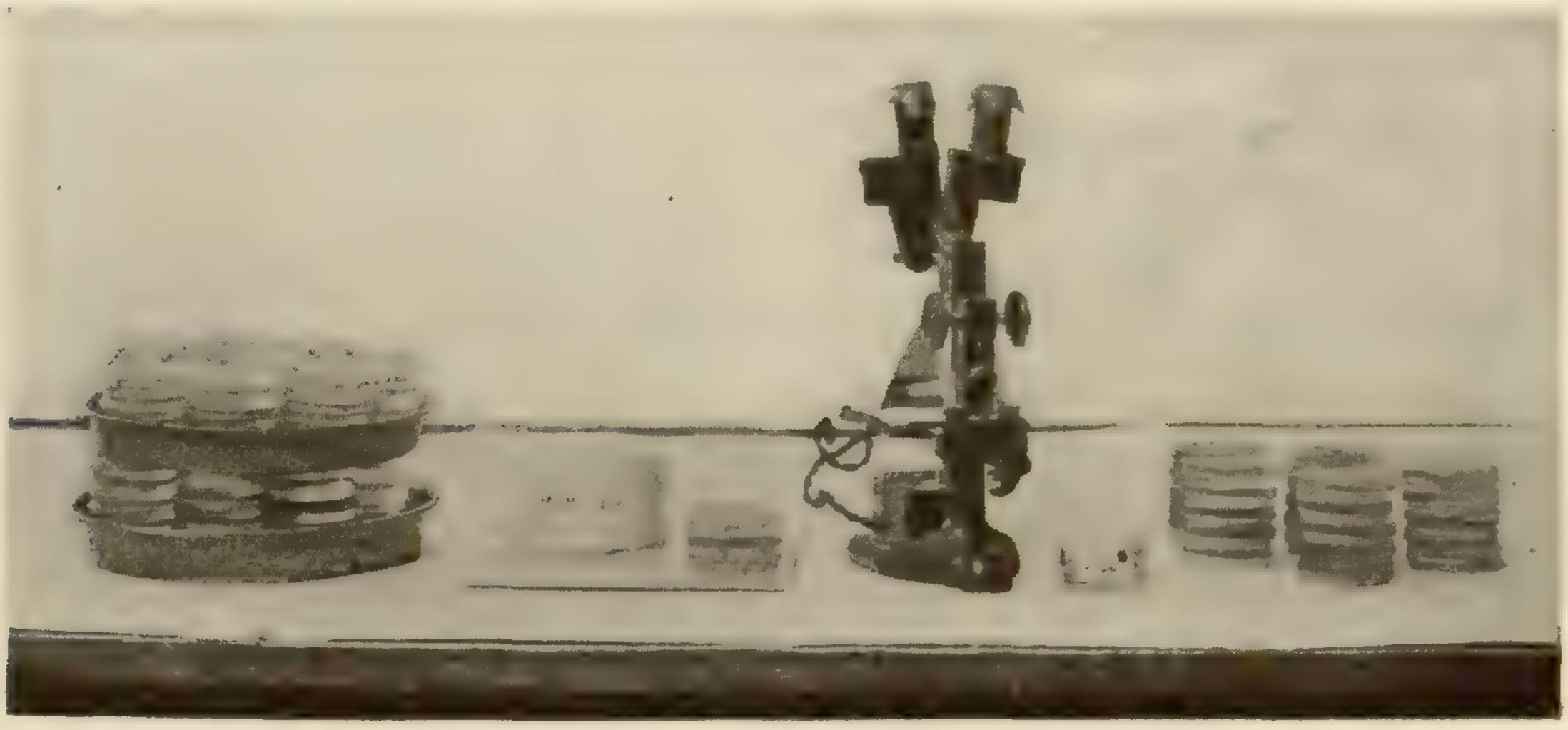

FIG. 2.—Culture dishes and apparatus used

stender dish marked "alcohol" contains a wad of cotton for swabbing the dishes. Blotters are seen on the stage of the binocular as well as the paper collar which partially incloses the culture under examination.

In earlier work on "imperfect hybridization" the bits of mycelium at the line of contact between two races were removed with needles and teased out for examination under the compound microscope in order to determine the presence or absence of sexual reactions. Unless stages in conjugation are relatively abundant, they are much more likely to be missed by this method than when examined at the proper time in the living condition under the binocular. The time of examination, however, must be well chosen, since if an examination is attempted after the line of contact between the two

races has become grown over, even a relatively strong reaction may not be evident. The growth of only a few hours may render a regrading of a culture impossible.

In the foregoing pages some of the sources of experimental error likely to be encountered in studying the sexual reactions between races of mucors have been pointed out, and the technique which a familiarity with these sources has led the writers to adopt has been outlined. What has been written may serve as an introduction to a paper to follow in this journal, in which it may be possible to show that the discordant conclusions reached by certain investigators working with the same material may have been due to differences in the methods employed.

STATION FOR EXPERIMENTAL EVOLUTION
COLD SPRING HARBOR, N. Y.

GERMINATION OF AECIOSPORES, UREDINIOSPORES, AND TELIOSPORES OF PUCCINIA CORONATA[1]

G. R. HOERNER

During comparatively recent years several workers have contributed considerable information concerning the phenomena of spore germination of crown rust of oats. The data herein recorded were the result of a series of experiments undertaken to determine: (1) the viability of aeciospores collected in several localities on species of *Rhamnus;* (2) the length of time urediniospores from a number of grass hosts, obtained in different localities, would remain viable; (3) the conditions under which urediniospores developed from artificial inoculations of oats in the greenhouse could retain their ability to germinate; (4) the optimum temperature for the germination of viable urediniospores; (5) whether teliospores developed on oat seedlings in the greenhouse would germinate immediately; and (6) how early in the spring teliospores produced in the field on a variety of hosts and collected in various places would germinate.

AECIOSPORE GERMINATION.[2]—From June 22 to July 11 inclusive specimens of rusted *Rhamnus* were collected at Montevideo and Moorehead, Minnesota; Wahpeton, North Dakota; Aberdeen and Brookings, South Dakota; and Beaver Dam, Wisconsin. Immediately following collection in the field, the fresh material was posted to Saint Paul, Minnesota, where the specimens were uniformly packeted in manila envelopes and filed in tin herbarium case boxes. The minimum period from the date of collection to the time the spore germination tests were made was 167 days. Negative results were attendant upon all attempts at germination of the aeciospores from any of the specimens.

[1] Investigations carried on while a graduate student at the University of Minnesota, 1916–1918.

[2] All spore germination tests in these experiments, unless otherwise stated, were made in hanging drops of distilled water in van Tieghem cells at room temperature (about 18°C.).

UREDINIOSPORE GERMINATION.—Rusted specimens of either *Avena fatua*, *A. sativa*, or *A. sterilis* were collected from May 7 to December 15 inclusive at San Diego and Santa Barbara, California; Carrol, Missouri Valley, Onawa, and Sioux City, Iowa; Lexington, Kentucky; Gilliam and Shreveport, Louisiana; Albert Lea, Belle Plaine, Caledonia, Granite Falls, Hinckley, Pipestone, Preston, Saint Paul, Sauk Center, Spring Valley, Two Harbors, Virginia, Wabasha, and Zumbrota, Minnesota; Sedalia and Springfield, Missouri; Pembina, North Dakota; Brookings, Bushnell, and Newell, South Dakota; Jackson, Knoxville, and Nashville, Tennessee; Beaumont and San Antonio, Texas; Lynchburg, Virginia, and Madison, Wisconsin. The freshly acquired material was treated in the same manner as were the specimens of the aeciosporic stage. After a maximum period of 87 days from date of collection, the urediniospores collected on *A. sativa* were still viable. To determine the possibility of differences in the germinating capacity of urediniospores secured on various hosts in different localities, when subjected to similar environmental conditions, the following series of greenhouse inoculations was devised:

a) Rusted specimens of *A. sativa* were collected at Saint Paul, Minnesota. The spores were used as the original inoculum for infecting Improved Ligowa Oats (Minn. 281). Spores obtained as a result of this infection were again used as a source of inoculum for another set of the same host. This procedure was continued for five successive spore generations.

b) Rust collected on *A. sterilis* at Saint Paul, Minnesota, was used as the original inoculum for infecting the same host as that mentioned in the preceding case. The subsequent procedure was the same.

c) The original inoculum for the third series was identical with that described in the second series. The successive inoculations resulted in two spore generations being developed on Improved Ligowa Oats (Minn. 281), one generation on *A. sterilis*, followed by two generations on Improved Ligowa Oats (Minn. 281).

d) Rusted specimens of *A. sterilis* were collected at Lynchburg, Virginia. The spores were used as the original inoculum. Subsequent inoculations resulted in five spore generations being devel-

oped on Improved Ligowa Oats (Minn. 281), one on *A. sterilis*, two on Improved Ligowa Oats (Minn. 281), followed by one generation on *A. sterilis*.

e) Rust collected on *A. sativa* at Lynchburg, Virginia, was used as the original inoculum. Subsequent inoculations resulted in five spore generations being developed on Improved Ligowa Oats (Minn. 281), one on *A. fatua*, followed by two generations on Improved Ligowa Oats (Minn. 281).

f) Rusted specimens of *A. fatua* were collected at San Diego, California. The spores were used as the original inoculum. Subsequent inoculations resulted in thirteen successive spore generations being developed on Improved Ligowa Oats (Minn. 281).

g) Rust collected on *A. sativa* at Tallulah, Louisiana, was used as the original inoculum. Subsequent inoculations resulted in thirteen successive spore generations being developed on Improved Ligowa Oats (Minn. 281).

Heavily rusted leaves were cut from plants in each series and placed in Petri dishes. Germination tests showed the spores from each series to be viable when removed from the greenhouse. The following environmental conditions were provided:

A. Petri dishes were placed outside and protected by covering with about one foot of leaves and snow.

B. Petri dishes were placed outside and not afforded any protection.

C. Petri dishes were wrapped in heavy manila paper and placed in a dark cabinet drawer, indoors.

D. Petri dishes were placed indoors fully exposed to sunlight.

Material from series *a*, *b*, *f*, and *g* was subjected to environment *A;* from series *a*, *e*, *f*, and *g* to environment *B;* from series *b* and *g* to environment *C;* from series *c* and *g* to environment *D*. Series *a*, *c*, *e*, *f*, and *g* under all of the environmental conditions and series *g* under environments *A*, *B*, and *D* gave no positive germination tests. The germination tests from series *b* under all environments provided, of series *d* under environment *A*, and of series *g* under environment *C* were all positive. The temperature range for environments out-of-doors was between $-27°$ F. and 42° F. inclusive, while for the indoors environments it was between 29° F. and

86° F. inclusive. No consistent differences as to viability of spores from various hosts and with different greenhouse life histories were noted.

Specimens of rusted *A. sativa* were collected at Saint Paul, Minnesota. Attempts were made to germinate spores at different temperatures, which resulted in positive germination tests at relatively low temperatures (about 7°C.), although apparently not above 32°C. A temperature of about 18°C. seemed to be the optimum.

JOHNSON,[3] in his germination studies of uredospores of crown rust of oats, came to similar conclusions. He gave 7°–8°C. as the minimum, 30°C. as the maximum, and 12°–17°C. as the optimum for germination.

TELIOSPORE GERMINATION.—Uredinial material collected from January 21 to April 20 on *A. sativa* and *A. sterilis* at Saint Paul, Minnesota, was used as the original source of inoculum from which sixteen different series of seedling oat hosts in the greenhouse were infected. Plants of each series produced telia in abundance. Negative results were obtained in all attempts to germinate these teliospores. Rusted specimens of either *A. sativa* or *A. sterilis* were collected from May 19 to May 2 of the year following, at Baton Rouge, Louisiana, and Olivia, Rochester, and Saint Paul, Minnesota. All attempts at germination gave negative results.

Summary

1. Aeciospores from herbarium specimens of *Rhamnus* were not viable after a period of 167 days from date of collection.

2. Urediniospores from herbarium specimens of *Avena sativa* proved to be viable as long as 87 days after date of collection.

3. Unprotected urediniospores lost their viability within 22 days, with a minimum temperature, during this period, of −27°F. and a maximum of 42°F.

4. When afforded protection with a temperature range similar to the unprotected, these spores remained viable as long as 44 days.

5. Exposed to light, viability of urediniospores was lost within

[3] JOHNSON, E. C., Cardinal temperatures for the germination of uredospores of cereal rust. Phytopath. 2:47, 48. 1912.

23 days, during which period the maximum temperature was 86° F. and the minimum 29° F.

6. Kept in the dark, urediniospores at similar temperatures to those exposed to light, remained viable as long as 79 days.

7. Urediniospores germinated at a temperature as low as 7° C., with an optimum of 18° C., and a maximum of 32° C.

8. Teliospores developed on oat seedlings in the greenhouse and not afforded a period of overwintering did not germinate.

9. Previous to overwintering and as late in the spring as May 2, teliospores developed in the field were incapable of germination.

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CURRENT LITERATURE

MINOR NOTICES

Handbook of Yosemite National Park.—HALL[1] has edited a volume of information in reference to Yosemite National Park. He has also written the chapter on trees, but the volume is really the combined product of more than a dozen specialists, and appears to be decidedly superior to the handbooks usually available for the guidance of travelers. The ideas and policy of actuating the best minds of the nation in the creation, administration, and use of such parks are admirably set forth by STEPHEN T. MATHER, Director United States National Park Service.

Several professors of the University of California have contributed chapters on their own subjects, KROEBER dealing with the anthropology, LAWSON with the geology, GRINNELL with the animal life and its distribution according to life zones, VAN DYKE with the insects, and JEPSON with the plant life. All these accounts are in attractive style and are scientifically accurate. JEPSON'S chapter on the *Sequoia* seems to be particularly happy in presenting the life problems of these great trees in attractive and accurate form. His treatment of the wild flowers is based upon ecological principles, and here, in common with the other chapters of the book, there is appended a sufficient bibliography to lead the interested traveler or student into the available literature upon the subject.—GEO. D. FULLER.

Plant analysis.—Stechert and Company have recently republished GREENISH'S translation of DRAGENDORF'S Plant analysis, qualitative and quantitative.[2]—WM. CROCKER.

NOTES FOR STUDENTS

Mutation.—For the past two decades the term mutation has held a very prominent place in the vocabulary and thought of biologists, yet most of us have had a very inexact understanding of the phenomena involved. Even now an understanding of the causes is probably quite remote, but at least our

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[1] HALL, ANSELL T., Handbook of Yosemite National Park. 12 mo. pp. ix+347. *pls. 27* and map. New York: Putman's Sons. 1921. $2.50.

[2] DRAGENDORF, G., Plant analysis, qualitative and quantitative. pp. vii+280. Translated by HENRY G. GREENISH. 1883.

knowledge of the characteristics of mutation are rapidly becoming more accurate. Mutations were first "discovered" by DE VRIES in *Oenothera Lamarckiana*, and characterized as being qualitative, discontinuous, and constant changes in the germ plasm. These three fundamental characteristics still hold true, but some of DE VRIES' other ideas on the subject have been considerably qualified by later work. For convenience the phenomena may tentatively be classified under five heads. Information has come largely from the published reports of a number of investigators, as indicated, and to some degree is supplemented by papers delivered at the last meetings of the American Association.

1. LOCUS CHANGE.—These are changes restricted to a single locus of one of the chromosomes. Usually they are effective only on one chromosome of a pair, without affecting the corresponding locus of its allelomorphic mate. Consequently the change first appears in the heterozygous condition. BAUR[3] estimates that such changes originate in the heterozygous condition 400 times as frequently as in the homozygous. These are mostly "loss" mutations and recessive to the previous condition. Only a very few dominant or "gain" mutations have been reported. This illustrates the trial and error method by which nature operates, only rarely making those gains which must serve as the basis for progressive evolution. In the fruit-fly these changes take place late in gametogenesis, since only one new individual of the new type appears in a progeny. BAUR, working on *Antirrhinum*, concludes that changes of this sort take place more frequently in the vegetative tissues than in connection with gametogenesis, which should result in large numbers of the new type among the progeny. In this respect it is quite probable that we are dealing with fundamentally different situations in animals, where the germ cells are differentiated so early in ontogeny, and in plants, where the germ cells are merely late products of permanent growing points.

ZELENY[4] states that there is no periodicity to these mutations, thus refuting one of the early ideas of DE VRIES. The same investigator demonstrates that reverse mutations are more frequent than original mutations. This, however, is simply because they are in the reverse direction, and not because of their recent origin. In the case of these reverse mutations, the changes are always full jumps back to the original starting point, and never result in an intermediate condition; nor will the selection of extreme types at all modify the rate at which these reverse mutations occur. In the opinion of the reviewer, however, this does not completely dispose of the possibility of modifying the rate of mutation by selection (it seems quite possible that rate of mutation might fall under the influence of multiple modifying factors).

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[3] BAUR, ERWIN, Mutationen von *Antirrhinum majus*. Zeit. Induct. Abstamm. Vererb. **19**:177–193. *figs. 10*. 1918.

[4] ZELENY, CHARLES, The direction and frequency of mutation in a series of multiple allelomorphs. Anat. Rec. **20**:210–211. 1921.

MULLER and ALTENBURG,[5] who have conducted a critical examination of the fruit-fly for mutations occurring on the first and second chromosomes, state that the vast majority of mutations have a lethal or semilethal effect when present in the homozygous (recessive) condition. It is obvious, therefore, that a critical search for mutations must involve a very special technique. MULLER is in possession of this technique through his intimate knowledge of the linkage groups on the chromosomes in question, and his ability to detect the absence of certain expected classes. On the sex chromosome he uncovered the startling fact that 50 per cent of the mutations were located in a restricted region at one end of the chromosome, which amounted to about 2 per cent of its length as charted from cross-over values. It is an open question whether this indicates a highly mutable region of the chromosome, or whether cross-over values are an inaccurate index of length.

The most promising phase of MULLER'S work arises from this critical study of the rate of mutation. Considering the whole length of the sex chromosome, one mutation occurs in 106 gametes. For the second chromosome the corresponding value is one in 175 gametes. ZELENY states that mutation is as frequent in one sex as in the other. Having established these constants, MULLER is now investigating the possibility of modifying the normal rate of mutation. Already he has been successful in depressing the rate one-half by means of low temperatures. Eventually such work may be of great practical value. A knowledge of the conditions necessary for the maximum rate of mutation should enable the pedigree culturalist to achieve much more rapid results than otherwise.

2. DEFICIENCY.—A rare phenomenon is described by BRIDGES,[6] working on the fruit-fly. This is more extensive than a simple locus change, being a "regional mutation," a loss or "inactivation" of a portion of a chromosome.

3. DUPLICATION.—BRIDGES describes another rare change, and other investigators have suspected similar phenomena. Some abnormal mitosis has resulted in the appearance of an extra piece of chromosome which duplicates in content a known region of the sex chromosome.

4. NON-DISJUNCTION.—This phenomenon, made famous through the classic work of BRIDGES on the sex chromosome of the fruit-fly, may prove to be a fairly common occurrence. In an irregular reduction division one of the chromosomes fails to "disjoin" properly from its mate. As a result, one or two gametes are formed with an extra chromosome, and others which lack this chromosome. The mating of one of the former with a normal gamete would produce a zygote with an extra chromosome. BLAKESLEE, BELLING,

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[5] MULLER, H. J., and ALTENBURG, E., A study of the character and mode of origin of eighteen mutations in the *X*-chromosome of *Drosophila*. Anat. Rec. **20**: 213. 1921.

[6] BRIDGES, CALVIN B., Vermilion-deficiency. Jour. Gen. Physiol. **1**:645–656. 1919.

and FARNHAM[7] have discovered this phenomenon in *Datura*. The normal diploid number of chromosomes in this form is twenty-four. Twelve different "mutants" have been discovered with twenty-five chromosomes. This seems to indicate that each of the twelve chromosomes (haploid) has failed to disjoin at least once in history. These twelve new forms are abnormal in their vegetative features, and notably low in fertility.

5. TETRAPLOIDY.—A hurried or incomplete mitosis will sometimes result in the simultaneous duplication of all of the chromosomes. This phenomenon has been observed several times, and there are indications that it has taken place frequently in the past. A general survey of the chromosome counts emphasizes the fact that the haploid number is much more frequently an even number than an odd one. This, together with the fact that there are several species groups in which the chromosome count of some of the members is just twice that of the others, suggests that tetraploidy may have played a considerable rôle in evolution. Tetraploidy commonly, but not always, brings gigantism.

BLAKESLEE now puts the finishing touches on this tetraploidy conception by more work on *Datura*. In addition to the abnormal forms with twenty-five chromosomes, he has discovered one completely triploid (thirty-six) and one tetraploid form. These latter both seem to be in a "better balanced" condition than the non-disjunctional (twenty-five) forms, since they are more "normal" with respect to their vegetative features and fertility. The beauty of the situation arises from the fact that the tetraploid type contains a previously known Mendelian factor. In normal forms a hybrid of the composition *Aa* will give a 3:1 ratio of purple- and white-flowered in the F_2. The tetraploid form *AAaa* gives gametes in the ratio 1 *AA*:4 *Aa*:1*aa*. These recombine to produce an F_2 of 35 purple:1 white. The F_3 and later generations behave according to expectations on this basis.

A question of terminology now arises. Of these various types of germinal changes, it seems the consensus of opinion to restrict the term mutation to the locus change. This is undoubtedly the most frequent type of change to take place, and possibly the most effective single factor in evolution. Deficiencies and duplications are very rare at best. Non-disjunction and tetraploidy are probably fairly common, and the latter is doubtless very important in evolution. These last two (and probably duplication as well) may be referred to collectively as "chromosome aberrations."

All of this differs from mutation as originally described by DE VRIES. This is not surprising in view of the fact that the original example of mutation was not a true case of mutation at all; it now seems certain that *O. Lamarckiana* is a hybrid, and its "mutants" merely recessives being segregated out.

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[7] BLAKESLEE, ALBERT F., BELLING, JOHN, and FARNHAM, M. E., Chromosomal duplication and Mendelian phenomena in *Datura* mutants. Science **52**:388–390. 1920.

MULLER[8] deserves the credit for solving this vexing problem. In the fruit-fly he discovered an essentially true-breeding hybrid race, and explained it by a system of balanced lethal factors. These factors assert their lethal effect only when they occur in the homozygous recessive condition. In this race of flies, two such factors are present in heterozygous condition on the same pair of chromosomes, the dominant members of the heterozygous sets being on the opposite chromosomes of the pair. Such a hybrid continues to breed true as such, since any attempt to segregate brings the homozygous recessive condition of one or the other lethal with resulting death to the progeny. The recessives of any heterozygous set on this same chromosome pair will remain concealed when the stock is allowed to inbreed. Occasional crossing-over will cause the appearance of a few (but in predictable frequencies) of these recessives, like the "mutants" thrown by *O. Lamarckiana*.

It is interesting to note that DE VRIES[9] himself now subscribes to an explanation which is fundamentally identical with the preceding. About one-half of the seeds of *O. Lamarckiana* are empty. DE VRIES explains by saying that *Lamarckiana* produces two kinds of gametes, the typical or this *laeta*, and the *velutina*. Each gamete has a lethal factor closely linked with the character factor. Heterozygous combinations give good seeds, homozygous give sterile. If one of the two lethal factors become "vital," the *O. laeta* or *O. velutina* mutation appears.—M. C. COULTER.

Taxonomic notes.—Miss BURLINGHAM[10] has described five new species of *Russula* from Vermont and one from Massachusetts, most of which seem to be rare.

SCHLECHTER[11] has revised two African genera of the Orchidaceae, *Schizochilus* and *Brachycorythis*. In the former genus he recognizes twenty-five species, thirteen of which are new; while in the latter genus twenty-three species are recorded, four of which are new. He also establishes two new genera, *Gyaladenia* and *Diplacorchis*.

MURRILL,[12] in continuation of his investigation of Polypores, has published an account of some of the resupinate forms which are rose-colored, lilac, red, or purple. He presents twenty-six species of *Poria*, five of which are

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[8] MULLER, H. J., Genetic variability, twin hybrids, and constant hybrids, in a case of balanced lethal factors. Genetics 3:422–499. *fig.* 1. 1918.

[9] DE VRIES, H., Phylogenetische und gruppenweise Artbildung. Flora 11–12: 208–226. 1918.

[10] BURLINGHAM, GERTRUDE S., Some new species of *Russula*. Mycologia 13: 129–134. *pl.* 7. 1921.

[11] SCHLECHTER, R., Revision der Gattungen *Schizochilus* Sond. und *Brachycorythis* Ldl. Beih. Bot. Centralbl. 38:80–131. 1921.

[12] MURRILL, WILLIAM A., Light-colored resupinate Polypores. III. Mycologia 13:83–100. 1921.

described as new. In a later publication[13] he considers the resupinate forms in which yellow is the predominant color, presenting sixteen species of *Poria*, seven of which are new.

BLAKE[14] has revised four genera of Compositae (Asteraceae) which are restricted in their distribution to the tropical and subtropical portions of North and South America, as follows: *Acanthospermum* (eight spp., three new), *Flourensia* (twenty-three spp., five new), *Oyedaea* (twelve spp., three new), and *Tithonia* (ten spp.). The revisions include full bibliography and lists of collections.

KAUFFMAN[15] has described a new genus (*Isoachlya*) of Saprolegniaceae, which is chiefly distinguished "by the presence of the cymose or *Achlya* mode of formation of secondary sporangia, coupled with diplanetic zoospores." It includes three species, one of which is new, the other two being transferred from *Achlya* and *Saprolegnia*.

NAKAI[16] has published a detailed monograph of the Caprifoliaceae of Japan, including 7 genera, 91 species, and 33 varieties. Besides the 15 new species, there are numerous transfers involving new names.

KUDO[17] has published an enumeration of the Labiatae of the Kurile Islands and Yezo Island, with full bibliography and citation of collections. The list includes 38 species, distributed among 21 genera. A new species is described in *Teucrium* and in *Scutellaria*.

BRITTON and ROSE[18] have described a new genus (*Neoabbottia*) of Cactaceae, a treelike form previously named *Cactus paniculatus* Lam., and later *Cereus paniculatus* DC. It is a monotypic genus of Hispaniola, dedicated to Dr. W. L. ABBOTT.—J. M. C.

Physical properties of protoplasm.—SEIFRIZ[19] has carried out microdissection of protoplasm from a number of lower animals and plants, and his work leads to the following conclusions. (1) There is a plasma membrane on

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[13] ———, Light-colored resupinate Polypores. IV. Mycologia **13**:171–178. 1921.

[14] BLAKE, S. F., Revisions of the genera *Acanthospermum*, *Flourensia*, *Oyedaea*, and *Tithonia*. Contrib. U.S. Nat. Herb. **20**:383–436. *pl. 23*. 1921.

[15] KAUFFMAN, C. H., *Isoachlya*, a new genus of the Saprolegniaceae. Amer. Jour. Bot. **8**:231–237. *pls. 13, 14*. 1921.

[16] NAKAI, TAKENOSHIN, Tentamen systematis Caprifoliacearum Japonicarum. Jour. Coll. Sci. Tokyo **43**: art. 2. pp. 139. 1921.

[17] KUDO, YUSHUN, Enumeratio Labiatarum specierum varietatum formarumque in Insulis Kurilensibus et Insula Yezoensi sponte nascentium. Jour. Coll. Sci. Tokyo **43**: art. 8. pp. 59. *pls. 2*. 1921.

[18] BRITTON, N. L., and ROSE, J. N., *Neoabbottia*, a new cactus genus from Hispaniola. Smithson. Miscell. Coll. **72**: no. 9. *pls. 1–4*. 1921.

[19] SEIFRIZ, W., Observations on some physical properties of protoplasm by aid of microdissection. Ann. Botany **35**:269–296. 1921.

the surface of all protoplasm; (2) physical considerations lead to belief in a differential surface layer of protoplasm; (3) plasma membrane differs in physical properties and probably in chemical constitution from the protoplasm it bounds; (4) the membrane is of high viscosity, probably a gel, which readily reverts to a liquid sol state; (5) it is capable of ready adjustment to changes in contour and area; (6) protoplasm in most cases forms a membrane almost instantly on the surface; exceptions are due to extreme liquidity; (7) the living membrane is rather delimited from the inner plasma, but it cannot be isolated from it; (8) the degenerated, coagulated plasma membrane can sometimes be isolated, being then of finer consistency, elastic, and exceedingly tough; (9) the nucleus and vacuoles also possess protoplasmic membranes resembling the outer plasma membrane; (10) the thickness of the membrane is probably about 0.1 μ.

Protoplasm, when dissected in water, in most cases is immiscible in it. When it is miscible, it is caused by extreme liquidity or disintegration. The immiscivity is possibly due to the colloidal and chemical nature of the protoplasm. The absorption and retention of water by protoplasm are essentially inhibition processes.—Wm. Crocker.

Food storage in cotyledons.—Duggar[20] has found that removal of the cotyledons of the pea seedling at an early stage of growth causes a much slower development of the plant, but their removal after the food is largely withdrawn causes no reduction in growth rate. Removal of the endosperm of the corn has far less effect. Glycocoll and sodium nucleinate in water culture partially substitute for the loss of the cotyledons. Asparaginate and alanin depress the growth with cotyledons removed. The author is to run experiments in sterile conditions to further test the possibility of organic materials substituting for the cotyledons.—Wm. Crocker.

Disease resistance.—McLean[21] concludes that Szinkum mandarin is resistant to citrus canker because its stomata are of such shape as to exclude liquid water and thus stop the entrance of the motile bacterium that produces the canker. The Florida seedling grapefruit which is susceptible to this disease has stomata of about the same size, but they are of such shape as to permit the accumulation of liquid water in the stomata and allow the entrance of the bacterium.—Wm. Crocker.

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[20] Duggar, B. M., The nutrition value of food reserve in cotyledons. Ann. Mo. Bot. Gard. **7**:291–298. 1920.

[21] McLean, F. T., A study of the structure of the stomata of two species of *Citrus* in relation to the citrus canker. Bull. Torr. Bot. Club **48**:101–106. 1921.

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SEXUAL DIMORPHISM IN CUNNINGHAMELLA

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(WITH ONE FIGURE)

Introduction

HETEROTHALLIC AND HOMOTHALLIC FORMS

In 1904 (1) it was shown that the mucors can be classified into two main groups according to their ability to produce zygospores from the sowing of a single spore. Species which are able to form sexual spores by the conjugation of branches from the same plant were called homothallic, since the mycelia appeared to be sexually alike; those which were able to form sexual spores only by the interaction of different plants were called heterothallic, since the mycelia taking part in conjugation appeared to be sexually different. The terms homothallic and heterothallic were used instead of hermaphroditic and dioecious because at the time they were first suggested our knowledge of sexuality in the mucors did not seem to warrant unreservedly accepting the idea of a strict sexual dimorphism in these forms, although such a dimorphism was strongly indicated by the interaction which had just been discovered between plus and minus races.

In later publications (**5, 6**) the desirability was pointed out of extending the use of the terms homothallic and heterothallic to signify the type of sexual differentiation in all gametophytes, in contrast with the terms homophytic and heterophytic suggested

for sporophytes. It was not expected that these terms would supplant the more familiar words hermaphroditic and dioecious. They may be found useful in bringing about greater accuracy in sexual terminology, and in emphasizing the inconsistency of calling a form like *Marchantia* dioecious and a form like the common lily hermaphroditic, when the two belong to the same sexual type.

Evidence for sex intergrades in heterothallic species

It is well known that sex intergrades are relatively frequent in sporophytic plants like the willow and hemp, which are commonly classified as dioecious. Similar sexual abnormalities, therefore, have been expected and sought for in heterothallic mucors ever since heterothallism was discovered in these forms in 1903. The fact that no race of a heterothallic mucor[1] has been found by the writers which, if it gave any sexual reaction at all, behaved otherwise than as a plus *or* a minus, indicates that sex intergrades in these forms are at best extremely rare. It is true that a number of investigators have reported findings which they have interpreted as opposed to the existence of a strict sexual dimorphism in the bread molds and related forms. Despite the fact that their conclusions were in harmony with our expectations based upon the condition in higher forms, the instances of supposed sex intergrades in heterothallic species are either isolated observations of two conjugating filaments which seem to originate from the same hypha, or have been supported by experimental evidence open to criticism. The inadequacy of the evidence has been pointed out in earlier publications (**4, 7, 8**), but it seems appropriate to mention two examples more or less typical of their kind. The first case is cited by Miss McCormick (**15**), and is illustrated by a figure showing a partially matured zygospore with the suspensor on one side arising from a filament which curves over and connects with the filament from the suspensor on the other side. In reply to an inquiry in regard to this zygospore Miss McCormick has written that after the

[1] The peculiar homothallic mycelium produced by regeneration of the germ tube and at times produced from the germination of spores in the germ sporangium in *Phycomyces* (**3**) is a temporary condition only, and apparently should more properly be considered a mixochimera of plus and minus protoplasm, as Burgeff (**13**) suggests, than as a sexually constant race.

drawings were made the homothallic strand was lost in an attempt to make a permanent mount of the fresh material. So far as we are aware, such a condition as Miss McCormick figures has not been described elsewhere for *Rhizopus* since 1903, when heterothallism was first discovered in the mucors. Inasmuch as an enormous number of zygospores of *Rhizopus* must have been observed more or less closely during this time, since it is a common type for laboratory study (Miss McCormick herself [**16**] reports having examined over 2000 in her cytological investigations in this species), it appears reasonable that in an isolated instance of this kind, the filaments from the two sides of the zygospore which appeared to be connected may in fact have been separate, but the place of separation obscured by overlying hyphae. That it is unsafe to judge of the thallic condition of a species from the hyphal connections is shown by experience with a class of students who were studying *Rhizopus* shortly after heterothallism had been discovered in other mucors, but before it had been demonstrated for this species. They were asked to find cases in which both the suspensors originated from branches of the same filament. A number of cases were found in which the two suspensors actually seemed to be connected, but in every instance there were one or more overlying filaments which would render the condition open to doubt by a critical observer.

The second case is a paper by Namyslowski (**17**), in which he throws doubts upon heterothallism in the whole group of the mucors. His experimental evidence comes from isolating single spores of *Rhizopus* and sowing the resulting mycelia on bread. The appearance of zygospores in fourteen out of forty-six such single spore cultures led him to conclude that his *Rhizopus* was homothallic. As has already been pointed out (**7**), the facts that six of these cultures were destroyed by bacteria and that thirteen more were devoid of even sporangia and hence probably also infected with bacteria, rendered it probable, to one familiar with pure culture methods, that the zygospores which Namyslowski obtained in part of his cultures from the sowing of a single spore actually might have arisen through interaction with the opposite sex which had gained access to these cultures through infection. This explanation seemed later confirmed by the isolation of the two sexual strains

from zygosporic material which NAMYSLOWSKI kindly sent to one of us. NAMYSLOWSKI (18), however, still believes, upon evidence which we have criticized, that heterothallic species have been shown capable of producing homothallic zygospores.

The two examples given are typical of less careful observations. Although cases of homothallism in heterothallic species on a priori grounds were to be expected, we have never found them ourselves, and could not feel that the reports of them by other investigators could stand critical examination. It reminded one of the reports of the birth of a full black negro baby from pure white parents which from time to time have appeared in literature and been passed on by rumor, but which have in no case been confirmed by students of human heredity.

BURGER ON CUNNINGHAMELLA

The condition outlined was the situation in the early part of 1919, when BURGER announced the finding of hermaphroditic as well as "pseudo-heterothallic" strains in *Cunninghamella* and *Syncephalastrum*. At the same time report came from one of the laboratories of the Department of Agriculture of a strain of *Rhizopus* which would form zygospores with both plus and minus test strains of this species. As to the *Rhizopus*, it was found upon inquiry that this particular strain had died out, and that after all it had not shown the capacity of conjugating with both the opposite sexes. BURGER'S paper on sexuality in *Cunninghamella* (14) presents the most extensive evidence which has yet appeared for sex intergrades in any heterothallic mucors. Although his arguments from the data presented seemed open to some criticism, his publication made the genus *Cunninghamella* the most likely source known for sex intergrades, the investigation of which would have considerable genetic interest. Recently published studies by one of us (11) had shown that races with plus and races with minus tendencies can arise by mutation from a homothallic species, and that such a race may cease to form zygospores and take on the appearance of a heterothallic species. It seemed worth while, therefore, to look for races with homothallic tendencies among heterothallic species, in view of BURGER'S paper. Accordingly, a rather extensive study of the interaction of strains

has been made for four species of *Cunninghamella*, the details of which will be given later. Since they offer no support to BURGER's conception of hermaphroditism in the genus, and since his cultures were allowed to die out before his final results were published, making it impossible for his material[2] to be retested, it is necessary to subject both his experimental technique and conclusions to searching criticism in lieu of other means of judging of the correctness of a statement which runs counter to the experience of most careful workers on the Mucorineae. BURGER's paper will be considered before discussing the results of this investigation.

BURGER found great irregularity in the sexual behavior of races of *C. bertholletiae*. While some races were consistently either plus or minus in reaction, others appeared to react both as plus and minus with properly chosen test strains. Certain races seemed to form a sexual triangle. His race *A*, for example, would form zygospores with *B*, race *B* would form zygospores with *C*, race *C* would form zygospores with *A*, and the family triangle was complete.

BURGER's conclusions are based primarily upon tests with twenty-six[3] races of *C. bertholletiae*. Since he says "authentic cultures of *C. bertholletiae* and *C. elegans* were obtained from Holland," and later credits us with having sent the only race of *C. elegans* which he used in his tests, there is little doubt that his race no. 21 of *C. bertholletiae* is identical with the no. 213 which we secured from the Centralstelle, and of which we sent a subculture to the Harvard laboratory with *C. elegans* shortly before BURGER used the strains in his investigations. In addition to these two races, he used the plus and minus strains of *C. echinulata* and of *Mucor V*, which had also been sent by us to the Harvard laboratory. The sexual races of these two species were contrasted with all his twenty-six races of *C. bertholletiae*, but without finding any "imperfect hybridization" reactions. The race of *C. elegans* and six races of *C. bertholletiae* were individually contrasted with the remaining races of a collection consisting of twenty-six races of *C. bertholletiae*, five races of *C. echinulata* (including our plus and minus strains),

[2] Except his race no. 21, which will be discussed later.

[3] In two places in the text (probably through error), his race no. 25 is called *Mucor V* minus.

and plus and minus strains of *Mucor V* and the plus strain of *C. elegans*. The positive and negative results are assembled in a table. The sexual condition seemed to BURGER so hopelessly confused that he was led to the following conclusions contained in his summary:

> 1. In *Cunninghamella* there does not exist sexual dimorphism.
> 2. *C. echinulata* plus and minus, or *Mucor V* plus and minus as separated by BLAKESLEE, are unable to form progametes or gametes when contrasted with any one of twenty-six cultures of *C. bertholletiae*.
> 3. Many of these cultures of *C. bertholletiae* were able to form zygospores when contrasted with certain other cultures of this same species.
> 4. There exists a selective power in some strains to form zygospores with certain other strains. This condition of pseudoheterothallism cannot be explained at present.
> 5. There exists a condition in some strains which might be called hermaphroditism.
> 6. In none of the hermaphroditic strains did branches of the same hyphae conjugate.
> 7. Zygospores were produced only when two strains were contrasted whose gametes were compatible.

It will be well to examine this summary to see whether the rather sweeping conclusions are warranted from BURGER'S own data, assuming for the moment that these data are not open to criticism. The results of his contrasts are more readily compared if his table be rearranged as shown in table I. The six testers of *C. bertholletiae* are placed at the top, together with the plus race of *C. elegans* and of *Mucor V*, which were also used as testers. On the side, grouped according to sex, are placed the twenty-six races with which the testers were contrasted; *H* stands for imperfect hybridization, *Z* for zygospores. If the latter is inclosed in parenthesis, it indicates the occurrence of zygospores in a contrast where they would not be expected on the basis of a strict sexual dimorphism. No grades are given in table I, since none are presented in the original paper.

The sexual behavior of the twenty-six races of *C. bertholletiae* shown in the table I is not so badly mixed as even BURGER himself was apparently led to believe from his method of analyzing the data. He says: "Nos. 1 and 2 have always remained constantly

plus, while nos. 4–6, 12, 15, 18, 19, 22–26 were always minus, nos. 3, 7–11, 13, 14, 16, 17, 20, 21, ... however, have reacted with both the so-called plus and minus strains." This is a curious conclusion, that nos. 1 and 2 are constantly plus because they

TABLE I

REARRANGEMENT OF DATA IN BURGER'S TABLE I: *Cunninghamella bertholletiae*, 26 RACES (1–26); NUMBER OF COMBINATIONS POSSIBLE, 325; NUMBER OF COMBINATIONS MADE, 135; ABERRANT COMBINATIONS, 6; "PSEUDOHETEROTHALLIC HERMAPHRODITIC RACES," 3 TO 8; Z STANDS FOR OCCURRENCE OF ZYGOSPORES, H FOR OCCURRENCE OF "IMPERFECT HYBRIDIZATION" REACTION; PARENTHESES INDICATE THAT REACTION IS ABERRANT ON BASIS OF SEXUAL DIMORPHISM

Races contrasted		9 plus or plus and minus	10 plus	14 plus or plus and minus	34 [*C. elegans* plus]	33 [V plus]	21 minus or plus and minus	7 minus or plus and minus	3 minus or plus and minus
Plus or plus and minus	9...	.....	O	O	O	O	Z	Z	Z
Plus	10...	O	.....	O	O	O	Z	Z	Z
Plus or plus and minus	14...	O	O	.....	O	O	Z	Z	Z
Plus or plus and minus	16...	O	O	(Z)	O	O	Z	Z	Z
Plus	17...	O	O	O	O	O	Z	Z	Z
Plus or plus and minus	20...	(Z)	O	(Z)	O	O	Z	Z	Z
Plus	1...	O	O	O	O	O	Z	O	O
Plus	2...	O	O	O	O	O	Z	O	O
Plus	11...	O	O	O	O	O	O	O	Z
Minus or plus and minus	3...	Z	Z	Z	H	O	(Z)	O	.....
Minus or plus and minus	7...	Z	Z	Z	O	O	(Z)	.....	O
Minus	8...	Z	Z	Z	H	O	O	O	O
Minus	12...	Z	Z	Z	H	O	O	O	O
Minus or plus and minus	13...	Z	Z	Z	H	O	(Z)	O	O
Minus or plus and minus	21...	Z	Z	Z	H	O	.....	(Z)	(Z)
Minus	4...	Z	O	Z	O	O	O	O	O
Minus	5...	Z	O	O	O	O	O	O	O
Minus	6...	Z	O	O	O	O	O	O	O
Minus	15...	Z	O	O	O	O	O	O	O
Minus	18...	O	Z	O	O	O	O	O	O
Minus	19...	O	Z	O	O	O	O	O	O
Minus	22...	O	Z	O	O	O	O	O	O
Minus	23...	O	Z	O	O	O	O	O	O
Minus	24...	O	O	O	H	O	O	O	O
Minus	25...	O	O	O	H	O	O	O	O
Minus	26...	O	O	O	H	O	O	O	O
[V minus]	32...	O	O	O	H	Z	O	O	O

produced zygospores with a hermaphrodite (no. 21) and with no other race; while no. 11 is listed among those which have reacted with both the so-called plus and minus strains when it formed zygospores only with no. 3, which need not be considered other than as a good minus. BURGER further believed that there were twelve hermaphrodites, since he lists this number, including no. 11 among

those reacting with both plus and minus strains. Referring to table I, it will be seen that in only eight contrasts are zygospores found where under a strict sexual dimorphism they would not be expected. Two of the eight are duplicates, leaving only six different contrasts showing aberrant reactions. It is not necessary, however, to consider more than three races hermaphroditic to account for the aberrant results. These three hermaphrodites may be variously chosen. Race no. 21 has three aberrant reactions, which is the largest number shown by any race. Both races nos. 14 and 20 show two aberrant reactions each, and may be chosen with no. 21 to make up the three hermaphrodites. Since nos. 14 and 20 are both assumed to be hermaphrodites, the reaction between them ought not perhaps to be credited to both of these races. However the credit of aberrancy is adjusted between nos. 14 and 20, race no. 21 remains the one which gives the largest number of aberrant reactions, and therefore of all the twenty-six races it is the óne most surely shown by BURGER'S data to be a hermaphrodite. This no. 21 is the same as our no. 213, and is the only one of the twenty-six races which it has been possible to reinvestigate. Its sexual behavior will be discussed more fully later.

Conclusion no. 1 of BURGER'S summary that in *Cunninghamella* there does not exist sexual dimorphism would seem too sweeping a statement in view of the fact that in *Mucor* and *Absidia*, which are predominantly heterothallic, forms are known, such as *Mucor heterogamus* (which with other similar species has been placed by some workers in the genus *Zygorhynchus*), and *Absidia spinosa*, which are homothallic. Races of two other species of *Cunninghamella* reported upon in the paper under discussion gave no evidence of hermaphroditism, and in consequence the data presented warrant the conclusion at most in reference to a single species. That in this single species three out of twenty-six races showed, in 135 out of a possible 325 contrasts, six reactions which were interpreted as indicating hermaphroditism, would render the species in the same class with the willows and others of the flowering plants called dioecious. That sexual dimorphism, strictly speaking, does not exist in higher plants is strongly suggested by past observations and experimentation, but the term sexual dimorphism is

currently applied to the so-called dioecious condition in forms like the willow, despite the familiar exceptions.

Conclusion no. 2, that the sexual races of *C. echinulata* and *Mucor V* as separated by us are unable to form progametes with any of the twenty-six races of *C. bertholletiae* studied, is too sweeping a statement, and will be shown later to be incorrect. In place of "are unable to form" should have been written "have not been observed to form" progametes.

The statements of fact in conclusions nos. 3, 4, and 7 are what one could make in regard to a heterothallic species. Conclusion no. 5, that a condition of hermaphroditism exists in some strains, seems somewhat opposed to the fact brought out in no. 6, that these hermaphrodites do not themselves take part in conjugation when growing alone in pure cultures, "a fact which indicates that this species is not homothallic," according to BURGER. "Homothallic" it will be remembered is a term used by us to indicate a hermaphroditic condition in gametophytes. The line of reasoning is as follows: some strains are hermaphrodites, in none of the hermaphroditic strains did branches of the same hyphae conjugate, therefore the species is not hermaphroditic.

Earlier in the paper the fact that the stock tubes containing the individual twenty-six races did not produce zygospores under nutrient and temperature conditions favorable for their formation showed according to BURGER "that the cultures were pure and not a mixture of strains." On the contrary, BURGER's own data show that lack of zygospore formation cannot be a proof of freedom from mixture of strains. Table I makes the matter clear. The minus race no. 4 fails to form zygospores with the plus race no. 10. If nos. 4 and 10 were mixed in a tube culture, therefore, they would not be expected to form zygospores, and yet the plus component (no. 10) of this mixture would form zygospores with the minus races 3, 7, 8, etc., while the minus component (no. 4) would form zygospores with the plus races nos. 9 and 14. The tube containing the mixture suggested would be able to conjugate, therefore, with both plus and minus strains, and such a reaction is BURGER'S proof of hermaphroditism in *Cunninghamella*. Table I shows that eighteen out of the twenty-six races could be mixed to form twenty different

combination pairs capable of reacting with both plus and minus races. If all of the 325 possible contrasts had been made between the twenty-six races instead of only the 135 actually attempted, it is probable that a considerably larger number of pairs of races, capable when mixed of producing zygospores with both plus and minus races, would be evident. So far as the data of BURGER go, however, they are sufficient to show that absence of zygospores in a culture cannot be offered as proof that it is not a mixture of strains; and to indicate that infection, if it occurred, rather than the existence of pseudoheterothallic hermaphrodites, might be the cause of zygospores in cultures where they would not be expected on the basis of sexual dimorphism.

BURGER believed he had eliminated the possible objection that his cultures had been mixed by making several single spore cultures from each of the strains nos. 9, 3, and 21, and obtaining zygospores whenever such cultures from one of these strains were contrasted with those from either of the other two strains. The test, on the face of it, may appear to be a critical one, and in fact if only these three strains had been kept in cultivation they would now afford an opportunity of critically retesting data upon which BURGER'S theories are based. Since, however, these cultures were destroyed before the publication of his paper in which their peculiar behavior is described, it will be necessary to depend upon circumstantial rather than upon direct evidence. As seen from table I, nos. 9 and 3 may be considered good plus and minus races, and in consequence should be expected to give reactions when grown together. In consequence, interest centers rather in strain no. 21. This race it will be remembered gave the most aberrant reactions, and together with nos. 14 and 20 is able to account for all the evidence that can be brought forward in support of BURGER'S theory of pseudoheterothallism. No 21 is predominantly minus and so should be expected to give reactions with the plus strain no. 9. The abnormal reaction, therefore, is between nos. 21 and 3. The surprising thing about these tests is that apparently there were no controls. Each of the three single spore cultures of no. 3 were contrasted against each of the four single spore cultures of no. 21,

but nothing was said about control contrasts between the subcultures of no. 3, nor of contrasts between the subcultures of no. 21, nor is mention anywhere made of uninoculated controls to discover what the danger might be from air infection of spores of the opposite sexes. For aught we know, single spore subcultures of any race might have appeared to produce zygospores when contrasted together at the time BURGER made his single spore cultures, which was apparently at the end of his series of contrasts with the twenty-six races. Neither in these single spore culture contrasts nor in any of the others is the abundance of zygospores graded. A single zygospore or a limited number which might make the investigator suspicious of mixture of strains in his stock culture or of infection in his contrast culture apparently have been classified as of equal value with our grades *A* and *B*.

In a previous paper (12) attention was called to the peculiar danger of air borne infection of *Cunninghamella* when forms of this genus had previously been grown in the laboratory. *Cunninghamella*, it may be remembered, was first described as an *Oedocephalum*, a hyphomycetous genus with exogenous spores, but was later (2) shown to be a heterothallic mucor by the isolation of its sexual races and their combination to form zygospores. It has already been shown that another investigator who found zygospores in his cultures after planting the mycelia from single spores was apparently misled into a theory of hermaphroditism for *Rhizopus* on account of unsuspected infection of his cultures with sexual races of the same species. It seems reasonable to suspect that BURGER has fallen into a similar error, since he gives no evidence to the contrary, rather than to believe he has discovered a sexual condition unparalleled in the experience of other critical workers.

There are a number of perhaps minor matters in the body of BURGER'S paper, such as the use of the terms neutral and zygotactic, to which objection might be made. Enough has been said, however, to indicate that his data do not inevitably lead to his main conclusion of pseudoheterothallic hermaphroditism in *Cunninghamella*.

BURGER'S CONCLUSIONS COMPARED WITH NEW DATA

It will be remembered that of the cultures used by BURGER, *Cunninghamella echinulata* plus and minus, *C. elegans* plus, *Mucor V* plus and minus, and his race no. 21 of *C. bertholletiae* were obtained from us. These races are still running, and it has been possible therefore to compare their behavior with the observations of BURGER on the same material.

The second conclusion in his summary, to the effect that neither the sexual strains of our *C. echinulata* nor those of our *Mucor V* are able to form progametes with any one of twenty-six cultures of *C. bertholletiae*, is contrary to our experience. Table II shows

TABLE II

"IMPERFECT HYBRIDIZATION" BETWEEN RACES OF *Cunninghamella bertholletiae* AND *Mucor V* PLUS AND MINUS; *Mucor V* PLANTED 4:00 P.M., 11/19/19; *C. bertholletiae* PLANTED 11:00 A.M., 11/20/19; RECORDS TAKEN 2:00 TO 4:00 P.M., 11/22/19; NUTRIENT NO. 380 (BURGER'S OATMEAL AGAR); c AND d INDICATE GRADES OF IMPERFECT HYBRIDIZATION; O INDICATES ABSENCE OF OBSERVED REACTION

C. bertholletiae	Plus races						Neutral races		Minus races					
	217	227	268	234	464	456	215	452	266	457	459	213	241	180
Mucor V plus...	O	O	O	O	O	O	O	O	c	c	O	d	c	O
Mucor V minus..	c	d	c	c	c	O	O	O	O	O	O	O	O	O

the results of contrasts between the sexual races of *Mucor V* and testers from the collection of races of *C. bertholletiae* grown on oatmeal agar made up according to BURGER'S method of preparation. The majority of the races (including no. 213, which is BURGER'S no. 21) showed "imperfect hybridizations" with the opposite sex of *Mucor V*. The nutrient chosen does not appear to be the best for the reaction, but was used to make the conditions of the experiment so far as possible comparable with those reported in the paper under discussion. "Imperfect hybridization" between *Mucor V* plus and our race no. 213 has been obtained on other nutrients, but the reaction with this particular race has never been strong and might readily have been missed had we employed a less successful method of observation (**12**).

Our old test races of *C. echinulata* (nos. 885 and 886) are able to form "imperfect hybridization" reactions with a number of the races of *C. bertholletiae*, although no sexual reaction between them and our race no. 213 has been observed.

Our race no. 213, which is the same as BURGER'S no. 21 and is the strain which furnished the strongest evidence for his theory of pseudoheterothallic hermaphroditism, has been tested against eighty-eight other races of the same species obtained from Brazil nuts from various localities. In all these tests it has reacted, if at all, only as a minus. In BURGER'S experience, although predominantly a minus, it produced zygospores in three combinations with the seventeen other minus races, a total of 17.5 per cent of the contrasts between it and other minus races. If it had reacted in the same manner we should have expected it to produce zygospores with a minimum of eleven of our sixty-eight other minus races. As a matter of fact, it showed reactions with none of these minus races. That so great a difference really exists between our minus strains and those studied by BURGER seems unlikely.

BURGER seems not to have observed the imperfect sexual reactions between races of *C. bertholletiae* which failed to carry through to zygospore formation. Partly for this reason perhaps, despite his own evidence already adduced to the contrary, he failed to appreciate the fact that absence of zygospore formation in a culture is not a proof of its freedom from mixture with strains of the opposite sex. The imperfect reactions in *C. bertholletiae* will be discussed later. In our experience the plus race no. 465 forms only imperfect reactions with the minus race 457. In consequence, when these two races are planted mixed in a Petri culture in contrast with the plus race no. 217 and the minus race no. 459, a triangular reaction has been obtained, as shown in fig. 1, where the mixture is represented as forming zygospores with *both* the plus race no. 217 and the minus race no. 459, while the latter two are also forming zygospores together. Although we have obtained the triangular reaction shown in fig. 1, which BURGER considered a proof that sexual dimorphism does not exist in *Cunninghamella*, we know in this case that the reaction is due to a mixture of strains and not to pseudoheterothallic hermaphroditism. BURGER'S

conclusions, therefore, are not justified from his own data, and the few races which it has been possible to retest from among those studied by him have shown either reactions which he considered impossible or have failed to show the reactions which he found and upon which his theory of pseudoheterothallic hermaphroditism in *Cunninghamella* was based. It must be emphasized, however, that despite the necessity for considering the evidence for sex intergrades in heterothallic mucors open to serious criticism, there is no proof at hand that such intergrades do not exist. A somewhat detailed consideration of the evidence for them in *Cunninghamella* has been given to indicate a few of the dangers into which even one with some experience with cultural methods is likely to fall. The data already published (**10**) and to be presented in the following pages show that sex intergrades must be extremely rare in the mucors, and place the burden of proof on observers who think they have found evidence for their occurrence.

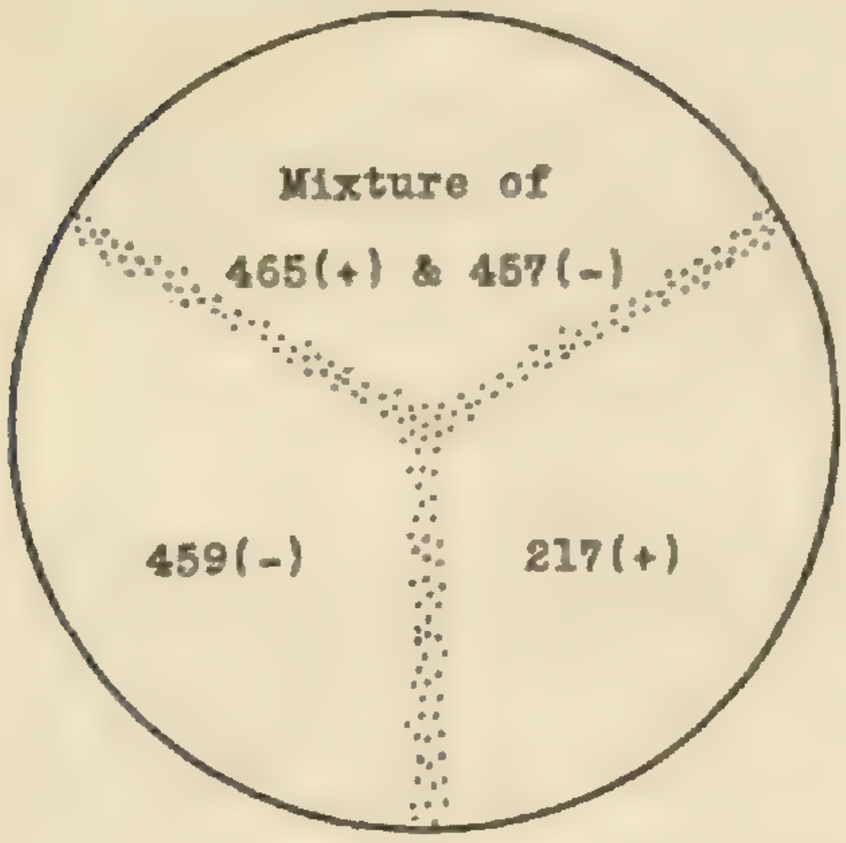


FIG. 1.—Diagram representing Petri dish culture: at lower right and left were planted respectively plus and minus races 217 and 459 which are forming zygospores (represented by dots at line of contact between them); in upper third was planted a mixture of plus and minus races 465 and 457 which fail to form zygospores with each other, but form them with the respective opposite sexes 459 and 217.

New data on Cunninghamella

Tests of the sexual condition in *Cunninghamella* were made with 202 races of four species; forty-two races of *C. elegans*, eighteen races of *C. echinulata*, eighty-nine races of *C. bertholletiae*, and fifty-three races of a species as yet unidentified.[4] The method of running the

[4] The discrepancy between the number of races given here and that listed in a previous publication (**10**) is due to the separation of *Cunninghamella A* from the other species and the omission of four races from the tables on account of infection in the stock tubes, because of incomplete records or for other reasons. All told, including tests with other genera, a considerably larger number of contrasts has been made with *Cunninghamella* than is reported.

gross cultures to obtain the races to be investigated, as well as the detailed methods of making contrasts between them has been described in a previous publication (**12**) and need not be repeated here. Table III gives the origin of the different races used in the tests. Samples of different types of soil were taken from different stations, chiefly near Cold Spring Harbor, and were the source of races of *C. elegans* and *C. echinulata*. Brazil nuts furnished both *C. bertholletiae* and *C. echinulata*, as well as the undetermined species *A*. All the gross cultures were given a serial number preceded by the letter *T* or *H*. The individual nuts in these cultures were indicated by capital letters, and the same was done for the spots on the soil and bread cultures from which transfers were made. In some cases more than a single transfer was made from an individual nut, as is shown by nos. 737 and 738. Generally more than a single race was isolated from each gross culture which showed fruits of the fungus sought, since, as table III shows, sexually distinct races are frequently present in the same gross culture. Undoubtedly among our numbered races some are duplicates, but duplication would probably not have been avoided if only a single race had been taken from each purchased collection of nuts.

More races of a single species were taken from *T*117 than from any other gross culture. From this culture, however, both plus and minus sexes were obtained, and the various races of the same sex are far from all being duplicates, as may be seen by comparing the records of nos. 732, 733, and 739, shown in table VII *A*. Despite the facts that the opposite sexes were frequently found to be present in the same culture and that the gross cultures were run at a temperature favorable for sexual reproduction, no zygospores of *Cunninghamella* in gross culture were found. Their absence may be due to the relatively meager growth of the fungus under the conditions in gross cultures.

The tests with the different species may be considered separately. The individual and mean grades were assigned as already described (**10**, **12**). For the most part, individual contrasts were made only once, since it seemed more profitable to obtain somewhat roughly graded records of a relatively large number of separate

TABLE III

LIST OF RACES OF *Cunninghamella* INVESTIGATED SHOWING RACE NUMBER; SPECIES, WHETHER *C. bertholletiae* (*C. berth.*) *C. echinulata* (*C. ech.*), *C. elegans* (*C. eleg.*), OR THE UNDETERMINED SPECIES *C.A.*; IN CASE OF BRAZIL NUTS, PLACE IN WHICH NUTS WERE PURCHASED IS INDICATED.

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
179..	C. berth.	H1 E	Brazil nuts	Huntington, N.Y.	x	.....	.....
180..	C. berth.	H1 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
181..	C. A.	H1 C	Brazil nuts	Huntington, N.Y.	x	.....	.....
182..	C. A.	H1 E	Brazil nuts	Huntington, N.Y.	.....	.....	x
183..	C. berth.	H1 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
184..	C. A.	H1 G	Brazil nuts	Huntington, N.Y.	.....	.....	x
185..	C. berth.	H1 A	Brazil nuts	Huntington, N.Y.	.....	.....	x
186..	C. berth.	H1 D	Brazil nuts	Huntington, N.Y.	.....	.....	x
187..	C. A.	H2 C	Brazil nuts	Huntington, N.Y.	.....	.....	x
188..	C. A.	H2	Brazil nuts	Huntington, N.Y.	.....	.....	x
189..	C. berth.	H2 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
190..	C. A.	H2 B	Brazil nuts	Huntington, N.Y.	x	.....	.....
191..	C. A.	H2 C	Brazil nuts	Huntington, N.Y.	x	.....	.....
192..	C. berth.	H2 C	Brazil nuts	Huntington, N.Y.	.....	.....	x
193..	C. A.	H2 E	Brazil nuts	Huntington, N.Y.	x	.....	.....
213..	C. berth.	.....	.....	"Centralstelle," Holland	.....	.....	x
214..	C. berth.	T12 C	Brazil nuts	New York City	.....	.....	x
215..	C. berth.	T13 Y	Brazil nuts	New York City	.....	x	.....
216..	C. berth.	T27 A	Brazil nuts	Huntington, N.Y.	.....	.....	x
217..	C. berth.	T38 A	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
218..	C. berth.	T38 D	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
219..	C. berth.	T38 E	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
220..	C. berth.	T39 A	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
221..	C. berth.	T39 B	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
222..	C. berth.	T39 D	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
223..	C. berth.	T40 A	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
224..	C. A.	T40 D	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
225..	C. A.	T40 B	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
226..	C. berth.	T40 C	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
227..	C. berth.	T40 E	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
228..	C. berth.	T48 A	Brazil nuts	Hicksville, N.Y.	.....	.....	x
229..	C. ech.	T13 G	Brazil nuts	New York City	x	.....	.....
232..	C. berth.	T50 F	Paradise nuts	New York City	.....	.....	x
233..	C. berth.	T51 A	Brazil nuts	New York City	.....	.....	x
234..	C. berth.	T51 B	Brazil nuts	New York City	x	.....	.....
245..	C. A.	T51 E	Brazil nuts	New York City	x	.....	.....
236..	C. ech.	T51 H	Brazil nuts	New York City	x	.....	.....
237..	C. berth.	T52 A	Brazil nuts	New York City	.....	.....	x
238..	C. ech.	T52 C	Brazil nuts	New York City	x	.....	.....
239..	C. A.	T52 D	Brazil nuts	New York City	.....	.....	x
240..	C. berth.	T53 B	Brazil nuts	Worcester, Mass.	.....	.....	x
241..	C. berth.	T53 C	Brazil nuts	Worcester, Mass.	.....	.....	x
242..	C. A.	T53 D	Brazil nuts	Worcester, Mass.	x	.....	.....
243..	C. berth.	T54 A	Brazil nuts	Washington, D.C.	.....	.....	x
244..	C. berth.	T54 B	Brazil nuts	Washington, D.C.	.....	.....	x
245..	C. berth.	T55 A	Brazil nuts	Brooklyn, N.Y.	.....	.....	x

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
246..	C. berth.	T55 B	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
247..	C. berth.	T55 F	Brazil nuts	Brooklyn, N.Y.	.....	x	.....
248..	C. ech.	T66 A	Brazil nuts	New York City	x	.....	.....
249..	C. berth.	T66 B	Brazil nuts	New York City	.....	x	.....
250..	C. ech.	T66 C	Brazil nuts	New York City	.....	.....	x
252..	C. berth.	T67 A	Brazil nuts	New York City	.....	.....	x
253..	C. berth.	T67 C	Brazil nuts	New York City	.....	.....	x
254..	C. berth.	T68 A	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
255..	C. ech.	T68 A	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
256..	C. A.	T68 B	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
257..	C. A.	T68 C	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
258..	C. berth.	T68 D	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
259..	C. A.	T68 E	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
260..	C. A.	T68 E	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
261..	C. A.	T73 A	Brazil nuts	New York City	x	.....	.....
262..	C. berth.	T73 B	Brazil nuts	New York City	.....	.....	x
265..	C. ech.	T44 B	Soil	Cold Spring Harbor	.....	.....	x
266..	C. berth.	T50 F	Paradise nuts	New York City	.....	.....	x
267..	C. A.	T52 D	Brazil nuts	New York City	x	.....	.....
268..	C. berth.	T52 D	Brazil nuts	New York City	x	.....	.....
269..	C. A.	T68 C	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
270..	C. A.	T27 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
271..	C. berth.	T38 B	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
272..	C. A.	T68 D	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
273..	C. A.	T73 C	Brazil nuts	New York City	x	.....	.....
274..	C. berth.	T73 C	Brazil nuts	New York City	.....	.....	x
275..	C. berth.	T74 E	Brazil nuts	New York City	.....	.....	x
372..	C. berth.	T76 B	Brazil nuts	New York City	.....	.....	x
373..	C. berth.	T76 C	Brazil nuts	New York City	.....	x	.....
446..	C. berth.	.....	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
447..	C. berth.	T74 C	Brazil nuts	New York City	.....	.....	x
448..	C. berth.	T75 A	Brazil nuts	New York City	.....	.....	x
449..	C. berth.	T79	Brazil nuts	New York City	.....	.....	x
450..	C. berth.	T80 B	Brazil nuts	New York City	.....	x	.....
451..	C. berth.	T80 E	Brazil nuts	New York City	.....	.....	x
452..	C. berth.	T81 B	Brazil nuts	Brooklyn, N.Y.	.....	x	.....
453..	C. berth.	T81 E	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
454..	C. berth.	T96 A	Brazil nuts	Storrs, Conn.	.....	.....	x
455..	C. A.	T96 E	Brazil nuts	Storrs, Conn.	.....	.....	x
456..	C. berth.	T96 C	Brazil nuts	Storrs, Conn.	x	.....	.....
457..	C. berth.	T97 B	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
458..	C. berth.	T99 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
459..	C. berth.	T99 D	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
460..	C. berth.	T99 C	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
461..	C. berth.	T100 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
462..	C. berth.	T101 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
463..	C. berth.	T101 B	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
464..	C. berth.	T101 C	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
465..	C. berth.	T98	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
466..	C. eleg.	T29 D	Soil	Cold Spring Harbor	x	.....	.....
467..	C. eleg.	T36 B	Soil	Cold Spring Harbor	.....	x	.....
468..	C. eleg.	T36 C	Soil	Cold Spring Harbor	.....	.....	x
469..	C. eleg.	T44 D	Soil	Cold Spring Harbor	x	.....	.....
470..	C. eleg.	T58 D	Soil	Cold Spring Harbor	x	.....	.....

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
471..	C. eleg.	T60 B	Soil	Cold Spring Harbor	x	.....	.....
472..	C. eleg.	T60 D	Soil	Cold Spring Harbor	.....	.....	x
473..	C. eleg.	T60 E	Soil	Cold Spring Harbor	x	.....	.....
474..	C. eleg.	T61 D	Soil	Cold Spring Harbor	x	.....	.....
475..	C. eleg.	T61 C	Soil	Cold Spring Harbor	.....	.....	x
476..	C. eleg.	T61 E	Soil	Cold Spring Harbor	.....	.....	x
477..	C. eleg.	T61 G	Soil	Cold Spring Harbor	.....	.....	x
478..	C. eleg.	T62 A	Soil	Cold Spring Harbor	.....	.....	x
479..	C. eleg.	T62 A	Soil	Cold Spring Harbor	.....	.....	x
480..	C. eleg.	T63 A	Soil	Cold Spring Harbor	.....	.....	x
481..	C. eleg.	T64 D	Soil	Cold Spring Harbor	. x	.....	.....
482..	C. eleg.	T64 C	Soil	Cold Spring Harbor	x	.....	.....
483..	C. eleg.	T64 E	Soil	Cold Spring Harbor	.....	.....	x
484..	C. eleg.	T64 F	Soil	Cold Spring Harbor	x	.....	.....
485..	C. eleg.	T65 C	Soil	Cold Spring Harbor	x	.....	.....
486..	C. eleg.	T65 A	Soil	Cold Spring Harbor	.....	.....	x
487..	C. eleg.	T84 A	Soil	Cold Spring Harbor	x	.....	.....
488..	C. eleg.	T84 B	Soil	Cold Spring Harbor	.....	.....	x
489..	C. eleg.	T84 D	Soil	Cold Spring Harbor	.....	.....	x
490..	C. eleg.	T85 B	Soil	Cold Spring Harbor	.....	.....	x
491..	C. eleg.	T85 D	Soil	Cold Spring Harbor	.....	.....	x
492..	C. eleg.	T85 E	Soil	Cold Spring Harbor	x	.....	.....
493..	C. eleg.	T86 A	Soil	Cold Spring Harbor	x	.....	.....
494..	C. eleg.	T86 C	Soil	Cold Spring Harbor	x	.....	.....
495..	C. eleg.	T86 E	Soil	Cold Spring Harbor	x	.....	.....
496..	C. eleg.	T86 F	Soil	Cold Spring Harbor	x	.....	.....
497..	C. eleg.	T86 G	Soil	Cold Spring Harbor	x	.....	.....
498..	C. eleg.	T87 B	Soil	Cold Spring Harbor	x	.....	.....
499..	C. eleg.	T87 F	Soil	Cold Spring Harbor	x	.....	.....
500..	C. eleg.	T89 A	Soil	Cold Spring Harbor	x	.....	.....
501..	C. eleg.	T89 B	Soil	Cold Spring Harbor	x	.....	.....
502..	C. eleg.	T89 C	Soil	Cold Spring Harbor	x	.....	.....
503..	C. eleg.	T92 B	Soil	Cold Spring Harbor	x	.....	.....
504..	C. eleg.	T92 D	Soil	Cold Spring Harbor	x	.....	.....
505..	C. eleg.	T92 D	Soil	Cold Spring Harbor	x	.....	.....
506..	C. eleg.	T92 E	Soil	Cold Spring Harbor	.....	.....	x
507..	C. eleg.	T92 G	Soil	Cold Spring Harbor	.....	.....	x
508..	C. A.	T52	Brazil nuts	New York City	x	.....	.....
510..	C. ech.	.....	Laboratory infection	Washington, D.C.	.....	.....	x
511..	C. A.	T73 D	Brazil nuts	New York City	.....	.....	x
512..	C. A.	T75 A	Brazil nuts	New York City	x	.....	.....
513..	C. A.	T75 B	Brazil nuts	New York City	.....	.....	x
514..	C. A.	T75 C	Brazil nuts	New York City	.....	.....	x
515..	C. A.	T75 F	Brazil nuts	New York City	x	.....	.....
516..	C. A.	T76 A	Brazil nuts	New York City	.....	.....	x
517..	C. A.	T76 D	Brazil nuts	New York City	.....	.....	x
518..	C. A.	T76 E	Brazil nuts	New York City	.....	.....	x
519..	C. A.	T79 B	Brazil nuts	New York City	.....	.....	x
520..	C. A.	T79 F	Brazil nuts	New York City	.....	.....	x
521..	C. A.	T80 A	Brazil nuts	New York City	x	.....	.....
522..	C. A.	T81 C	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
523..	C. A.	T81 G	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
524..	C. A.	T96 E	Brazil nuts	Storrs, Conn.	x	.....	.....

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
525..	C. ech.	T98 C	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
526..	C. ech.	T99 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
527..	C. ech.	T97 A	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
528..	C. ech.	T99 E	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
529..	C. ech.	T99 E	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
718..	C. berth.	T111 E	Brazil nuts	Norway, Me.	.....	.....	x
719..	C. berth.	T112 F	Brazil nuts	Parkersburg, W.Va.	.....	.....	x
720..	C. berth.	T113 C	Brazil nuts	Louisville, Ky.	.....	x	.....
721..	C. berth.	T114 A	Brazil nuts	Franklin, Ind.	.....	.....	x
722..	C. berth.	T114 D	Brazil nuts	Franklin, Ind.	.....	.....	x
723..	C. berth.	T115 A	Brazil nuts	Hickory, N.C.	.....	x	.....
724..	C. berth.	T115 B	Brazil nuts	Hickory, N.C.	.....	.....	x
725..	C. berth.	T115 B	Brazil nuts	Hickory, N.C.	.....	.....	x
726..	C. berth.	T115 F	Brazil nuts	Hickory, N.C.	.....	.....	x
727..	C. berth.	T116 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
728..	C. berth.	T116 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
729..	C. berth.	T116 G	Brazil nuts	Knoxville, Tenn.	.....	.....	x
730..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
731..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
732..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
733..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
734..	C. berth.	T117 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
735..	C. berth.	T117 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
736..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
737..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
738..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
739..	C. berth.	T117 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
740..	C. berth.	T118 A	Brazil nuts	Knoxville, Tenn.	.....	.....	x
741..	C. berth.	T118 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
742..	C. A.	T111 A	Brazil nuts	Norway, Me.	.....	.....	x
743..	C. A.	T111 B	Brazil nuts	Norway, Me.	.....	.....	x
744..	C. A.	T111 C	Brazil nuts	Norway, Me.	.....	.....	x
745..	C. A.	T111 G	Brazil nuts	Norway, Me.	.....	x	.....
747..	C. ech.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
748..	C. A.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
749..	C. A.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
750..	C. ech.	T113 C	Brazil nuts	Louisville, Ky.	.....	.....	x
751..	C. A.	T113 D	Brazil nuts	Louisville, Ky.	.....	.....	x
752..	C. A.	T113 D	Brazil nuts	Louisville, Ky.	x	.....	.....
753..	C. A.	T116 A	Brazil nuts	Knoxville, Tenn.	.....	.....	x
754..	C. A.	T116 D	Brazil nuts	Knoxville, Tenn.	.....	x	.....
755..	C. A.	T116 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
756..	C. ech.	T116 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
757..	C. A.	T116 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
758..	C. A.	T116 H	Brazil nuts	Knoxville, Tenn.	.....	.....	x
759..	C. A.	T118 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
779..	C. berth.	T115 D	Brazil nuts	Hickory, N.C.	x	.....	.....
885..	C. ech.	.....	Laboratory culture	Cambridge, Mass.	x	.....	.....
886..	C. ech.	.....	Laboratory culture	Cambridge, Mass.	.....	.....	x

contrasts than to attempt to secure more accurate records by averaging the grades of a relatively few contrasts which had been several times repeated. If any of the cultures had become infected or in any other way appeared abnormal, the contrasts of course were repeated. In a few cases, especially in the earlier contrasts with *C. bertholletiae*, zygospores were found where, on the basis of a strict sexual dimorphism, they would not be expected. A repetition of these contrasts under improved technique gave the results incorporated in table VII A, and indicated that their earlier aberrant behavior was due to infection with the opposite sexes of the same species. All contrasts with species of *Cunninghamella* have been grown in the incubating oven at 24°–27°C.

Cunninghamella elegans

Table IV shows the tests with *C. elegans*. Twelve races were used as testers, and in all 426 contrast combinations were made with the total forty-two races. Of these, twenty-five were plus, sixteen were minus, and one, on account of its failure to show reactions in any of the combinations tested, has provisionally been listed as a neutral.

Cunninghamella echinulata

Table V shows the tests with *C. echinulata*. All the 153 possible contrast combinations were made with the total eighteen races. Of these, ten were plus, eight were minus, and none failed to show a sexual reaction in at least two contrast combinations. Since no reactions occurred when races with like sign were contrasted together, only the contrasts between plus and minus races are represented in the table.

Cunninghamella A

Table VI shows the tests with the undetermined species of *Cunninghamella* provisionally termed *Cunninghamella A*. It is a form intermediate in appearance between *C. bertholletiae* and *C. echinulata*, and was at first confused with them. In tube cultures it approaches more nearly the habit and color of *C. echinulata*. From this species, however, it may readily be distinguished microscopically, especially by the lack of conspicuous echinulations on the conidia. The form, however, needs a more careful study than

TABLE IV

SUMMARY OF TESTS OF *Cunninghamella elegans:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY LETTERS A TO D; ABSENCE OF ZYGOSPORES INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 230 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT DRY MALT EXTRACT, 2 PER CENT DEXTROSE, AND 0.1 PER CENT MEAT PEPTONE.

Grade	Races	Minus testers					Plus testers						
		475	472	468	478	507	496	466	474	469	470	471	473
	Plus races												
2.80	496	B	B	B	B	C	....	O	O	O	O	O	O
2.60	482	A	B	B	C	D	O	O	O	O	O	O	O
2.40	487	B	A	B	D	D	O	O	O	O	O	O	O
2.40	505	C	B	B	D	B	O	O	O	O	O	O	O
2.20	466	B	C	D	C	B	O	....	O	O	O	O	O
2.20	474	A	A	C	D	O	O	O	....	O	O	O	O
2.20	494	A	B	C	D	D	O	O	O	O	O	O	O
2.20	499	B	B	B	C	O	O	O	O	O	O	O	O
2.20	504	B	B	C	D	C	O	O	O	O	O	O	O
2.00	469	C	B	C	B	O	O	O	O	....	O	O	O
2.00	500	B	C	B	D	D	O	O	O	O	O	O	O
2.00	502	C	C	C	D	B	O	O	O	O	O	O	O
1.80	471	C	B	B	D	O	O	O	O	O	O	....	O
1.80	497	C	C	C	C	D	O	O	O	O	O	O	O
1.60	470	C	C	B	D	O	O	O	O	O	....	O	O
1.60	484	B	C	D	D	D	O	O	O	O	O	O	O
1.60	498	C	C	C	C	O	O	O	O	O	O	O	O
1.40	492	D	O	B	D	C	O	O	O	O	O	O	O
1.40	473	D	D	D	C	C	O	O	O	O	O	O	....
1.40	481	C	C	C	D	O	O	O	O	O	O	O	O
1.40	485	C	C	C	D	O	O	O	O	O	O	O	O
1.40	493	C	C	D	D	D	O	O	O	O	O	O	O
1.40	495	C	C	D	D	D	O	O	O	O	O	O	O
1.40	503	C	D	D	D	C	O	O	O	O	O	O	O
1.00	501	O	O	C	C	D	O	O	O	O	O	O	O
	Neutral races												
0.00	467	O	O	O	O	O	O	O	O	O	O	O	O
	Minus races												
2.71	506	O	O	O	O	O	B	C	B	B	B	B	C
2.57	472	O	....	O	O	O	B	C	A	B	C	B	D
2.57	476	O	O	O	O	O	B	B	B	B	C	B	D
2.57	480	O	O	O	O	O	B	B	C	C	A	B	D
2.43	475	....	O	O	O	O	B	B	A	C	C	C	D
2.29	479	O	O	O	O	O	C	B	C	C	C	B	C
2.29	489	O	O	O	O	O	B	B	C	C	C	C	C
2.14	468	O	O	....	O	O	B	D	C	C	B	B	D
2.14	488	O	O	O	O	O	B	C	B	C	C	C	D
2.14	490	O	O	O	O	O	B	C	C	C	C	C	C
1.86	478	O	O	O	....	O	B	C	D	B	D	D	C
1.86	483	O	O	O	O	O	A	C	D	C	D	C	D
1.71	491	O	O	O	O	O	C	C	C	C	C	D	D
1.71	486	O	O	O	O	O	B	C	C	C	C	O	D
1.00	507	O	O	O	O	....	C	B	O	O	O	O	C
0.71	477	O	O	O	O	O	O	D	D	D	D	D	O
Grades (all combinations)		2.36	2.24	2.12	1.44	1.12	2.69	2.25	2.12	2.06	1.94	1.94	1.31

it has received before it can justly be described as a distinct species. The manner in which the races reacted in combinations first suggested that another species was included in the collections, and a later inspection and microscopic examination showed that species

TABLE V

SUMMARY OF TESTS OF *Cunninghamella echinulata:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY LETTERS A TO D; ABSENCE OF ZYGOSPORES INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; CONTRASTS BETWEEN RACES OF SAME SEX MADE BUT NOT REPRESENTED; IN ALL CASES THEY FAILED TO PRODUCE ZYGOSPORES; NO. 362 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT WHEY POWDER, AND 1 PER CENT DEXTROSE.

		Grade									
		2.50	2.25	1.87	1.63	1.63	1.50	1.25	1.25	1.13	0.50
Grade	Minus races	Plus races									
		747	885	527	229	525	238	236	255	248	528
2.70	886.....	B	B	B	B	B	C	B	B	C	C
2.30	265.....	C	C	C	C	B	C	C	B	B	C
2.00	750.....	C	B	B	A	O	B	B	D	D	O
1.60	510.....	B	B	B	C	C	C	O	D	O	O
1.50	529.....	B	C	C	C	O	C	C	O	C	O
1.10	526.....	C	B	O	O	B	O	O	C	D	O
0.70	756.....	C	C	C	O	O	D	O	O	O	O
0.50	250.....	B	O	O	O	C	O	O	O	O	O

A could be distinguished from the other species. Six races were used as testers, and in all 297 contrast combinations were made with the total fifty-three races. Of these, twenty-two were plus, twenty-nine were minus, and two, on account of their failure to show reactions in any of the combinations tested, were listed as neutrals. Imperfect sexual reactions, indicated by small letters in table VI, will be discussed under the following species.

CUNNINGHAMELLA BERTHOLLETIAE

Table VII *A* shows the tests with *C. bertholletiae*. Fifteen races were used as testers, and in all 1215 combinations were made with the total eighty-nine races. Of these, twelve were plus, sixty-nine were minus, and eight, on account of their failure to show reactions

TABLE VI

SUMMARY OF TESTS OF *Cunninghamella A:* RELATIVE STRENGTH OF ZYGOSPORE FORMATIONS IN DIFFERENT COMBINATIONS INDICATED BY CAPITAL LETTERS A TO D; STRENGTH OF IMPERFECT REACTIONS BY SMALL LETTERS a TO d; ABSENCE OF SEXUAL REACTIONS INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 362 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT WHEY POWDER, AND 1 PER CENT DEXTROSE.

Grade	Races	Minus testers			Plus testers		
		269	182	257	515	242	181
	Plus races						
4.00	515	A	A	A	.....	O	O
3.67	242	A	A	B	O	.....	O
3.33	181	B	B	A	O	O	.....
3.00	191	B	B	B	O	O	O
2.67	759	C	B	B	O	O	O
2.33	267	C	C	B	O	O	O
2.33	748	C	B	C	O	O	O
2.00	261	C	C	C	O	O	O
2.00	755	C	C	C	O	O	O
1.67	235	C	C	D	O	O	O
1.67	259	C	C	D	O	O	O
1.67	508	C	D	C	O	O	O
1.67	521	D	C	C	O	O	O
1.67	749	D	C	C	O	O	O
1.33	260	C	D	D	O	O	O
1.33	273	C	D	D	O	O	O
1.33	524	D	C	D	O	O	O
1.00	190	D	D	D	O	O	O
1.00	512	D	O	C	O	O	O
0.67	522	D	D	O	O	O	O
0.33	752	D	O	O	O	O	O
0.33	193	D	O	O	O	O	O
	Neutral races						
0.00	745	O	O	O	O	O	O
0.00	754	O	O	O	O	O	O
	Minus races						
3.67	182	O	.....	O	A	A	B
3.67	257	O	O	.....	A	B	A
3.67	269	.....	O	O	A	A	B
3.33	517	O	O	O	A	b	b
3.33	523	O	O	O	A	b	b
3.00	744	O	O	O	A	C	B
3.00	455	O	O	O	A	C	B
2.33	514	O	O	O	A	D	C
2.33	256	O	O	O	B	C	C
1.67	224	O	O	O	B	D	D
1.67	188	O	O	O	d	b	D
1.33	513	O	O	O	B	O	D
1.00	239	O	O	O	B	O	O
1.00	751	O	O	O	b	O	O
0.67	184	O	O	O	C	O	O
0.67	270	O	O	O	C	O	O

TABLE VI—*Continued*

Grade	Races	Minus testers			Plus testers		
		269	182	257	515	242	181
	Minus races						
0.67	272	0	0	0	C	0	0
0.67	511	0	0	0	C	0	0
0.67	516	0	0	0	C	0	0
0.67	518	0	0	0	C	0	0
0.67	520	0	0	0	C	0	0
0.67	742	0	0	0	C	0	0
0.67	743	0	0	0	C	0	0
0.67	753	0	0	0	C	0	0
0.67	758	0	0	0	C	0	0
0.67	757	0	0	0	c	0	0
0.67	187	0	0	0	c	0	0
0.33	225	0	0	0	D	0	0
0.33	519	0	0	0	0	0	d
Grades (all combinations) .		1.91	1.86	1.82	2.59	0.97	1.03

in any of the combinations tested, have been provisionally listed as neutral.

C. bertholletiae seems to differ from the other species of *Cunninghamella* investigated except species *A*, and in fact from all the other mucors which have been studied in the same manner, in that between certain races imperfect sexual reactions have been found which do not lead to zygospore formation. It is possible that such reactions may occur more frequently than is realized. In contrasting the first few testers of a given species, the practice has been to look for imperfect reactions at an early stage of development, and, if none are found, to examine the culture dishes in later series only at the end of the growth period when imperfect reactions would not readily be recognized. It is thus possible that some of the zero records for the contrasts of species *A* in table VI would be replaced by grades of imperfect sexual reaction if they had all been retested and inspected at an early growth period. Imperfect reactions are graded in the tables by small letters instead of by the capitals used for zygospore formation. The reaction might readily be confused with the early stages of zygospore formation or the final stages of "imperfect hybridization." Imperfect hybridization has heretofore been found to occur only between the opposite sexes of different species. When dealing with races of

TABLE VII

SUMMARY OF TESTS OF *Cunninghamella bertholletiae:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY CAPITAL LETTERS A TO D, RELATIVE STRENGTH IN IMPERFECT SEXUAL REACTION INDICATED BY SMALL LETTERS a TO d; PRODUCTION OF PARTHENOSPORES (*a*-ZYGOSPORES) INDICATED BY CAPITAL LETTER FOLLOWED BY AN ASTERISK; ABSENCE OF OBSERVED SEXUAL REACTION INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR SEXUAL REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 230 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT DRY MALT EXTRACT, 2 PER CENT DEXTROSE, AND 0.1 PER CENT MEAT PEPTONE.

GRADE	RACES	*A.* INTRASPECIFIC REACTIONS															*B.* REACTIONS WITH SPECIES A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	452	217	227	268	234	464	456	188	455	269	191	515
	Plus races																				
3.00	217.....	A	B	B	B	b	b	C	O	O	...	O	O	O	O	O	d	c	C*	O	...
2.71	234.....	b	b	c	b	c	b	b	O	O	O	O	O	...	O	O	b	d	C*	O	O
2.71	268.....	B	A	B	B	c	c	c	O	O	O	O	...	O	O	O	c	b	C*	O	...
2.57	465.....	C	b	B	b	c	b	C	O	O	O	O	O	O	O	O	...	...	D*	O	...
2.57	227.....	B	C	B	B	c	b	c	O	O	O	...	O	O	O	O	c	...	C*	O	...
2.43	464.....	B	B	B	B	c	d	C	O	O	O	O	O	O	...	O	c	...	D*	O	...
2.14	738.....	B	C	B	A	D	O	c	O	O	O	O	O	O	O	O	...	...	...	O	...
1.71	179.....	b	c	D	d	b	C	O	O	O	O	O	O	O	O	O	...	...	C*	O	...
1.43	456.....	C	b	c	D	d	O	d	O	O	O	O	O	O	O	O	...	O	...	...	O	...
1.29	218.....	D	c	c	O	C	C	O	O	O	O	O	O	O	O	O	...	...	O	O	...
1.29	271.....	c	C	c	d	c	O	O	O	O	O	O	O	O	O	O	...	...	C*	O	O
0.43	779.....	D	O	D	O	O	D	O	O	O	O	O	O	O	O	O	...	...	...	O	...
	Neutral races																				
0.00	215.....	O	O	O	O	O	O	O	...	O	O	O	O	O	O	O	O	...	...	O	...
0.00	247.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	249.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	373.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	450.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	452.....	O	O	O	O	O	O	O	O	...	O	O	O	O	O	O	O	...	...	O	...
0.00	720.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	723.....	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
	Minus races																				
3.00	732.....	O	O	O	O	O	O	O	O	O	A	A	B	C	B	c	...	...	O	b	C*
3.00	266.....	...	O	O	O	O	O	O	O	O	A	B	B	b	B	C	O	O	...	d	...
3.00	274.....	O	O	O	O	O	O	O	O	O	B	B	B	b	A	c	...	...	O	c	...
3.00	729.....	O	O	O	O	O	O	O	O	O	A	A	A	O	A	c	...	...	O	c	...
3.00	457.....	O	...	O	O	O	O	O	O	O	B	C	A	b	B	b	O	O	...	c	...
2.83	737.....	O	O	O	O	O	O	O	O	O	A	B	B	c	B	c	...	...	...	c	...
2.67	213.....	O	O	O	...	O	O	O	O	O	B	B	B	b	B	D	O	...	O	c	...
2.67	252.....	O	O	O	O	O	O	O	O	O	B	B	B	c	B	c	...	...	...	b	...
2.67	262.....	O	O	O	O	O	O	O	O	O	A	B	B	b	B	O	...	...	...	O	...
2.67	459.....	O	O	...	O	O	O	O	O	O	B	B	B	c	B	c	O	...	O	c	...
2.50	731.....	O	O	O	O	O	O	O	O	O	B	B	B	C	B	d	...	...	...	a	D*
2.50	736.....	O	O	O	O	O	O	O	O	O	A	A	B	O	C	c	...	...	...	b	...
2.33	458.....	O	O	O	O	O	O	O	O	O	C	C	C	b	B	c	...	...	...	b	...
2.17	219.....	O	O	O	O	O	O	O	O	O	B	D	C	a	B	O	...	...	...	D	...
2.00	180.....	O	O	O	O	O	O	...	O	O	C	c	c	b	C	d	O	...	O	O	...
2.00	183.....	O	O	O	O	...	O	O	O	O	b	c	c	c	c	d	O	O	O	b	...

TABLE VII—*Continued*

Grade	Races	A. Intraspecific reactions															B. Reactions with species A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	452	217	227	268	234	464	456	188	455	269	191	515
	Minus races																				
2.00	241.....	O	O	O	O	O	...	O	O	O	b	b	c	b	d	O	O	O	O	c	...
1.67	186.....	O	O	O	O	O	O	O	O	O	b	c	c	b	O	O	...	...	...	O	...
1.67	244.....	O	O	O	O	O	O	O	O	O	b	b	c	c	O	O	...	...	...	b	...
1.50	192.....	O	O	O	O	O	O	O	O	O	d	c	c	c	c	O	...	...	...	O	...
1.50	216.....	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	O	...
1.50	223.....	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	c	...
1.50	226.....	O	O	O	O	O	O	O	O	O	c	c	c	c	d	O	...	...	...	O	...
1.50	232.....	O	O	O	O	O	O	O	O	O	c	c	c	O	B	O	...	...	...	c	...
1.50	233.....	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	O	...
1.50	451.....	O	O	O	O	O	O	O	O	O	b	c	c	O	O	c	...	...	...	O	...
1.33	221.....	O	O	O	O	O	O	O	O	O	c	c	c	c	O	O	...	...	...	d	...
1.33	258.....	O	O	O	O	O	O	O	O	O	C	c	c	d	D	O	...	...	...	O	...
1.33	448.....	O	O	O	O	O	O	O	O	O	d	c	c	d	O	c	...	...	...	b	...
1.33	463.....	O	O	O	O	O	O	O	O	O	b	c	c	d	O	O	...	...	...	O	...
1.33	733.....	O	O	O	O	O	O	O	O	O	c	O	d	d	c	c	...	...	...	b	...
1.17	185.....	O	O	O	O	O	O	O	O	O	D	c	c	d	d	O	...	...	...	b	...
1.17	189.....	O	O	O	O	O	O	O	O	O	d	c	c	c	O	O	...	...	...	O	...
1.17	222.....	O	O	O	O	O	O	O	O	O	d	c	c	c	O	O	...	...	...	c	...
1.17	243.....	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	O	...
1.17	275.....	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	b	...
1.17	460.....	O	O	O	O	O	O	O	O	O	B	c	c	O	O	O	...	...	...	c	...
1.17	461.....	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	O	...
1.17	718.....	O	O	O	O	O	O	O	O	O	B	d	O	O	C	d	...	...	...	O	...
1.00	220.....	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	228.....	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	254.....	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	446.....	O	O	O	O	O	O	O	O	O	d	c	c	d	O	O	...	...	...	a	...
1.00	449.....	O	O	O	O	O	O	O	O	O	d	c	c	d	O	O	...	...	...	c	...
0.83	214.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	240.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	246.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	447.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	453.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	454.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	462.....	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	741.....	O	O	O	O	O	O	O	O	O	c	d	O	O	O	C	...	...	...	d	...
0.83	372.....	O	O	O	O	O	O	O	O	O	d	O	O	a	O	O	...	...	...	O	...
0.67	253.....	O	O	O	O	O	O	O	O	O	c	c	O	O	O	O	...	...	...	b	...
0.67	722.....	O	O	O	O	O	O	O	O	O	d	O	O	O	b	O	...	...	...	c	...
0.67	726.....	O	O	O	O	O	O	O	O	O	c	O	O	O	c	O	...	...	...	c	...
0.67	730.....	O	O	O	O	O	O	O	O	O	c	O	d	O	d	O	...	...	...	O	...
0.67	735.....	O	O	O	O	O	O	O	O	O	B	O	O	O	d	O	...	...	...	d	...
0.67	740.....	O	O	O	O	O	O	O	O	O	c	O	O	O	c	O	...	...	...	c	...
0.50	721.....	O	O	O	O	O	O	O	O	O	d	O	O	O	O	C	...	...	...	c	...
0.50	734.....	O	O	O	O	O	O	O	O	O	c	O	O	O	d	O	...	...	...	c	...
0.33	237.....	O	O	O	O	O	O	O	O	O	O	O	c	O	O	O	...	...	...	O	...
0.33	245.....	O	O	O	O	O	O	O	O	O	d	d	O	O	O	O	...	...	...	O	...

TABLE VII—*Continued*

Grade	Races	*A.* Intraspecific reactions															*B.* Reactions with species A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	450	217	227	268	234	464	456	188	455	269	191	515
	Minus races																				
0.33	719	0	0	0	0	0	0	0	0	0	c	0	0	0	0	0	...	...	...	c	...
0.33	724	0	0	0	0	0	0	0	0	0	d	0	0	0	d	0	...	...	...	c	...
0.33	727	0	0	0	0	0	0	0	0	0	c	0	0	0	0	0	...	...	...	c	...
0.33	728	0	0	0	0	0	0	0	0	0	d	0	0	0	d	0	...	...	...	a	...
0.33	739	0	0	0	0	0	0	0	0	0	0	0	0	0	c	0	...	...	...	0	...
0.17	725	0	0	0	0	0	0	0	0	0	d	0	0	0	0	0	...	...	...	c	...
Grades (all combinations)		2.50	2.42	2.33	2.08	1.83	1.67	1.33	0.00	0.00	2.09	1.71	1.68	1.09	1.07	0.52					

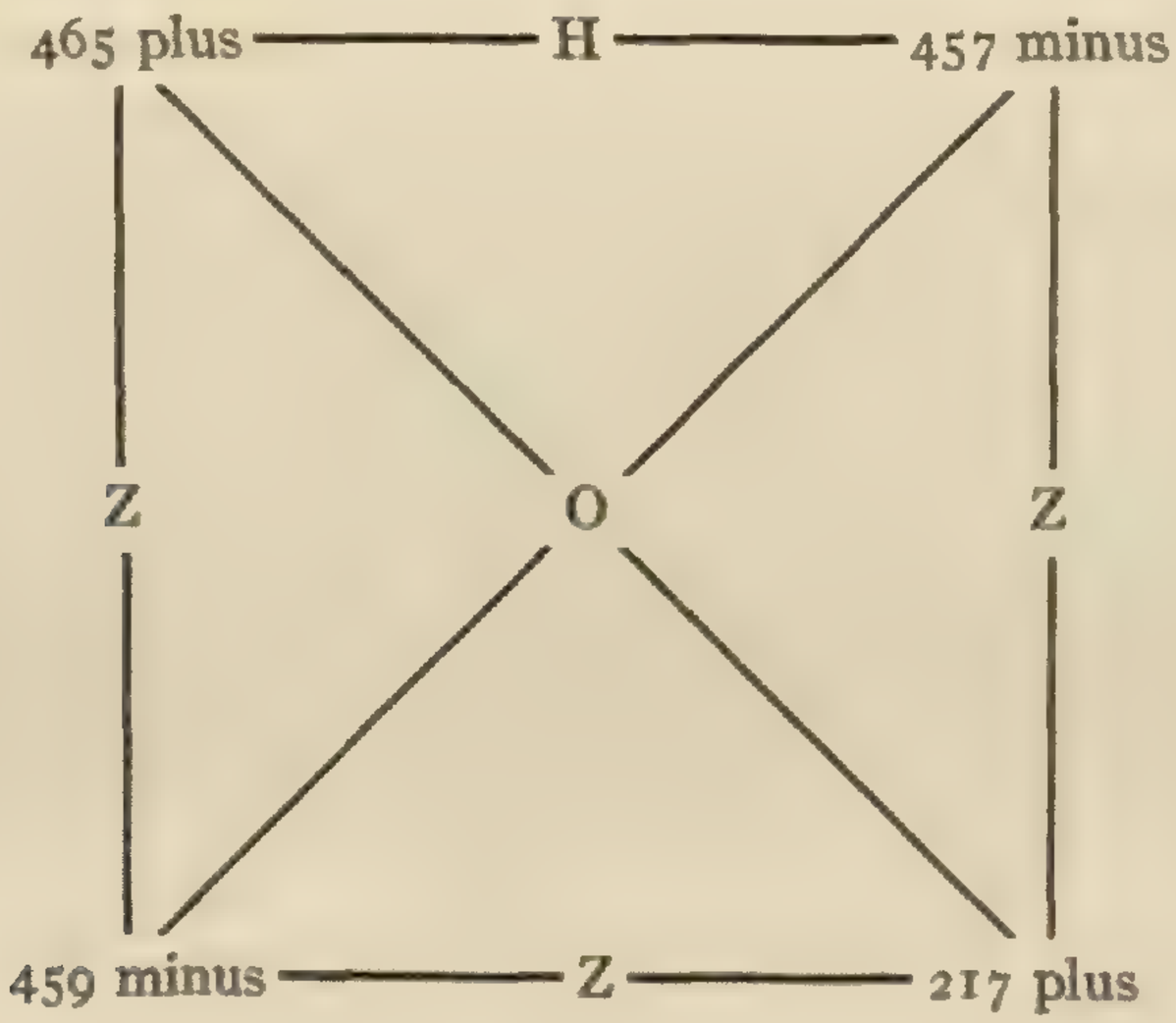
the same species, if any reaction was initiated at all, it was carried through to the production of zygospores, except of course for some obviously detrimental check in environmental conditions. The sexual process resulting in the production of zygospores may be considered the sum of two distinct reactions; first, the formation of opposed progametes or at most gametes; second, the dissolution of the cross walls between the gametes and the growth of the fusion cell into a zygospore. Only the first reaction can take place when the plus and minus races contrasted belong to different species.[5]

A number of facts indicate, however, that in the races listed under *C. bertholletiae* we are dealing with a single species. The essential uniformity of the strains in morphological appearance speaks for specific identity, and the production of zygospores fails to separate them into any consistent groups. A quadrangular reaction within selected groups of four may be discerned from table VII *A*. Thus the same four races shown in fig. 1 form the following quadrangle, in which *Z* stands for zygospore formation, *H* for imperfect sexual reaction, and *O* for no sexual reaction. Other similar quadrangles may be assembled from table VII *A*. The fact that only imperfect reactions are found in certain contrasts when

[5] True hybrids have been reported between closely related species of *Mucor* by Saito and Naganishi (**19**).

zygospores would be expected does not alter the sex of the races involved. When they take part in any sexual reactions at all, they are consistently either plus or minus. The first reaction of the sexual process is sufficient to indicate their sex, and gives an index of their sexual vigor. In calculating the mean grades of sexual activity for the different races, therefore, it has been considered fairest to give the imperfect reactions equal weight with zygospore formation.

What are the causes which prevent one combination in a quadrangular reaction from carrying the sexual process through to completion is a question requiring further study. In certain cases,



at least, the distance between the inoculations of opposing strains seems to be a matter of some importance. In *Circinella spinosa* it has always been necessary to inoculate the opposite sexes very close together in order to obtain zygospores, which are not formed beyond a few millimeters from the points of inoculation. In a few cases a retest of an imperfect reaction between races of *C. bertholletiae*, but with inoculations close together, has shown zygospore formation.

Certain contrasts which were repeated with inoculations at the usual distance apart gave different reactions from those first obtained, as may be seen by a comparison of table VII *A* with table VIII where the retest contrasts are listed. In table VIII there is, as might be expected, a certain amount of change in the grades assigned to the strength of the reactions. The present

interest, whoever, centers upon the grades inclosed in parentheses, which indicate reactions which have changed from a perfect to an imperfect reaction as shown by the production of stages resembling imperfect hybrids in place of zygospores. It will be seen that there are certain unexplained irregularities in the production of zygospores or of only imperfect reactions which indicate that the preliminary tests have not discovered all the factors involved. Enough has been learned, it is believed, to indicate that some of the factors are environmental which determine whether a sexual process in this species goes through to

TABLE VIII

RETESTS OF CONTRASTS BETWEEN RACES OF *Cunninghamella bertholletiae:* CAPITAL LETTERS INDICATE GRADES OF ZYGOSPORE FORMATION; SMALL LETTERS INDICATE GRADES OF IMPERFECT SEXUAL REACTION; LETTERS INCLOSED IN PARENTHESES SHOW CHANGE IN TYPE OF REACTION FROM ZYGOSPORE FORMATION TO IMPERFECT REACTIONS; REACTIONS WITH ASTERISK INDICATE PRESENCE OF PARTHENOSPORES; NO. 230 NUTRIENT USED.

Races	266	729	732	457	737	213	459	731	219	180	241	232	460	718	741	721
217			A					A		C		c	(O)	C		
234			(b)		c			(a)		c						
268										c			c			
465	C			b		b	B									
227									B							
179	c						C				(d)*					
456	C	c		c		C									(c)*	(c)*
218											C					
271	c			(c)												

completion with the formation of zygospores or is confined to the first reaction with the formation of progametes or at the most gametes.

Although environmental differences not readily controlled in the cultures may have some influence upon the extent of the sexual reaction, the genetic constitution of the individual races in the main must be responsible for their sexual behavior. We have not succeeded, however, in an attempt to subject the genetic differences to a factorial interpretation. Distinct classes of plus and minus races differing sharply in the strength of their sexual activity or in their capacity to form zygospores or only imperfect reactions with certain other races do not seem to exist. Thus certain contrasts from table VII *A* may be arranged in such a way that no

fewer than five differences in reaction are shown both in the plus and minus strains chosen to form table IX. A graded series is indicated which might indefinitely be expanded as more and more races were tested.

"Imperfect hybridization" between species

Tables IV to VII *A* deal with sexual reactions between races within the individual species concerned. In tables II, VII *B*, X, and XI are given the results of contrasting individual races of one species with those of another species. Many of the contrasts were made before the species *Cunninghamella A* was separated from

TABLE IX

Arrangement of selected races from table VII *A* showing graded differences in strength of reaction when contrasted: capital letters indicate grades of zygospores, small letters grades of imperfect sexual reaction.

Minus races	Plus races				
	217	227	456	779	234
266	A	B	C	D	b
459	B	B	c	D	c
213	B	B	D	O	b
457	B	C	b	O	b
180	C	c	d	O	b

C. echinulata and *C. bertholletiae*. Those between the testers *H* and *D* and the races of *Cunninghamella A* were made merely for the purpose of identifying the sex of the latter, and were not graded, since they were not originally intended for publication. It has seemed best, however, to include these and the reactions in table VII *B*, since they furnish cumulative evidence in regard to sexual dimorphism in *Cunninghamella*. Two races of *C. elegans* (nos. 496 and 506, respectively plus and minus) failed to show reactions with the old plus and minus testers of *C. echinulata* (nos. 885 and 886). In table VII *B* certain combinations are starred because in them the imperfect hybridization reactions led to the production of parthenospores (*a*-zygospores). When certain races of high sexual vigor are contrasted, gametes which have been formed but which have been unable to unite may develop into thick-walled sculptured

spores, which are with difficulty distinguished from the true zygospores. Superficial inspection under low magnifications would undoubtedly lead to their classification as zygospores, but it is not unlikely that in our records, especially the earlier ones on *C. bertholletiae*, contrasts may have been listed as weak zygospore reactions,

TABLE X

SUMMARY OF REACTIONS BETWEEN DIFFERENT SPECIES OF *Cunninghamella*: Z INDICATES ZYGOSPORES; SMALL LETTERS INDICATE GRADED IMPERFECT REACTIONS

	C. bertholletiae		C. echinulata		C. elegans	
	217 plus	266 minus	885 plus	886 minus	496 plus	506 minus
C. bertholletiae						
217 plus	.....	Z	O	c	O	c
266 minus	Z	.....	c	O	c	O
C. echinulata						
885 plus	O	c	.....	Z	O	O
886 minus	c	O	Z	.....	O	O
C. elegans						
496 plus	O	c	O	O	.....	Z
506 minus	c	O	O	O	Z	.....

TABLE XI

Cunninghamella A: UNGRADED "IMPERFECT HYBRIDIZATION" REACTIONS WITH PLUS AND MINUS MUCOR TESTERS *H* AND *D*; *H* IN BODY OF TABLE INDICATES IMPERFECT REACTIONS

Mucor	Plus races						Minus races						
	515	242	759	260	273	522	182	269	188	751	270	511	225
H plus	O	O	O	O	O	O	H	H	H	H	H	H	H
D minus	H	H	H	H	H	H	O	O	O	O	O	O	O

when they should have been called imperfect reactions with formation of parthenospores. A close examination, especially in the younger stages, will show that parthenospores develop from single gametes, and that the suspensor on only one side has a typical appearance, with what appears to be the suspensor on the opposite side frequently more or less rounded off and not closely adnate to the spore. The parthenospores themselves are often distinctly misshapen, but when the zygospores are small, as is true of those of species of *Cunninghamella*, it may be difficult to distinguish them

even with careful inspection. Parthenospores have been obtained between certain strong races of different species in other genera with larger zygospores when no doubt of their true nature was likely to occur after a careful examination. Figures of parthenospores formed on homothallic species, at stimulus of contact with a sexually vigorous race of a heterothallic species, are given in an earlier publication (**9**, *pl. I*). The possible presence of parthenospores must not be overlooked in judging reports (**19**) of true hybridization between different species in the mucors.

So far as the reactions between different species of *Cunninghamella* have been tested, they argue for the sexual dimorphism of this genus.

Discussion

The data in the present paper refer only to the mucor genus *Cunninghamella*. A preliminary summary has already been given of tests with other genera (**10**), and it is hoped to publish a detailed account of these tests at a later date. The data so far accumulated show no behavior inconsistent with the idea of a strict sexual dimorphism. The work, especially with *Cunninghamella*, indicates that sex intergrades must be extremely rare if ever present in these forms, despite the fact that they would be expected on a priori grounds and the fact that other observers have thought they had found them.

In the species of *Cunninghamella* there is apparent a graded series so far as the strength of sexual activity is concerned, ranging from a reaction with grade *A* between sexually strong races to grade *O* between sexually weak races. Races which have shown no reactions in any contrast tested are provisionally listed as "neutral." The term neutral is obviously relative, and not meant to indicate absolute absence of sex. The number of races listed as neutral for a given collection tends to decrease as more testers are used in contrasts. Thus it is evident from table VII *A* that if strain no. 217 had not been used as a tester, strains nos. 719, 727, and 725 would have been listed as neutrals rather than as minus strains, since they would have shown no reaction against any of the plus or minus testers used. Neutrals seem to form the low

extreme of a continuously graded series of sexual vigor, and the term as applied undoubtedly includes both plus and minus races.

It is doubtful whether much significance can be attributed to the proportion of plus and minus races in the collections of the different species of *Cunninghamella* as indicative of their relative distribution in nature. In *C. bertholletiae* the minus sex seems to greatly predominate over the plus. In *C. elegans* the condition is reversed. The first species was obtained from Brazil nuts bought in different stores, mostly in or around New York City. Many of the gross cultures, therefore, may have originated from the same wholesale shipments. The races may be representative of the shipments from which they came rather than of the locality where they were grown. Experience with *Rhizopus* (4) indicates that in a mixed culture which is producing zygospores in abundance, one is likely to isolate almost exclusively one or the other of the two sexes. The cargo carriers from which the nuts originated may have been infected chiefly with minus strains. That there is considerable diversity in sexual vigor of these strains, however, is seen from the tables. *C. elegans* was obtained from different types of soil around Cold Spring Harbor, and it is possible that collections from other regions would show a predominance of the opposite sex.

The clearest result from the study of *Cunninghamella* is the fact that in 2091 contrasts (2250 including contrasts between different species of *Cunninghamella*) made between 202 races from four different species (see footnote 4) there were none which, if they showed any sexual response at all, reacted otherwise than as either a plus or a minus.

Miss ALICE M. PRICKET, Miss MARGARET CONOVER, and Miss MARY E. DRUMMOND have assisted in the progress of the investigation which is here reported.

Summary

1. The terms heterothallic and homothallic are distinguished as applied to gametophytic sexual differentiation in the mucors.

2. Types of the evidence in support of sex intergrades in heterothallic mucors are given and criticized.

3. BURGER'S paper on *Cunninghamella*, in which he concludes that sexual dimorphism does not exist in this genus, is discussed (1) from the standpoint of his own data, (2) from the standpoint of our experience, and the decision is reached that his conclusion is not warranted.

4. Data on *Cunninghamella elegans*, *Cunninghamella A* (an undetermined species), *C. echinulata*, and *C. bertholletiae* give a total of 2250 contrasts between a total of 202 races.

5. In *C. bertholletiae* certain contrast combinations lead to imperfect sexual reactions when zygospores might be expected.

6. In none of the species were races found which reacted as sex intergrades.

7. It is concluded that so far as the material investigated is concerned *Cunninghamella* is sexually dimorphic.

STATION FOR EXPERIMENTAL EVOLUTION
COLD SPRING HARBOR, N.Y.

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NOTES ON WILLOWS OF SECTIONS PENTANDRAE AND NIGRAE

CARLETON R. BALL

(WITH FOUR FIGURES)

In 1905 the writer began a series of contributions under the title, *Notes on North American Willows*, of which three were published.[1] This general title has been dropped because of the great disadvantage of not being able to indicate clearly, in the title, the content and scope of each paper. For this reason the most recent contribution appeared under a specific title,[2] as does the present one. These data have been derived from studies incident to the treatment of the genus *Salix* in various floras and manuals of botany.[3]

The location of the herbarium specimens cited is as follows: B, herbarium C. R. BALL; C, Canadian Geological Survey, Ottawa; D, herbarium C. C. DEAM, Indiana; F, Field Museum, Chicago; FBb, Bebb Herbarium in Field Museum; I, Iowa State Agricultural College; N, United States National Herbarium; N.D., North Dakota Agricultural College; N.M., New Mexico Agricultural College; R, Rocky Mountain Herbarium, University of Wyoming.

SALIX SERISSIMA (Bailey) Fernald.—*S. arguta** *S. pallescens* Anderss. Svensk Vetensk. Acad. Handl. **6**:32. 1867.—*S. lucida serissima* Bailey in ARTHUR, Bull. Geol. Nat. Hist. Survey Minn.

[1] BOT. GAZ. **40**:376–380. *pls. 12, 13.* 1905; **60**:45–54. *figs. 3.* 1905; and **60**: 391–399. 1915.

[2] BALL, C. R., Undescribed willows of the section Cordatae. BOT. GAZ. **71**: 426–434. *fig. 1.* 1921.

[3] BALL, C. R., *Salix* in COULTER and NELSON, Man. Bot. Rocky Mt. Region, pp. 128–139. 1909.

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3:19. 1887.—*S. serissima* (Bailey) Fernald, Rhodora 6:7. December 28, 1903.

When this species was established by FERNALD, in the very interesting and comprehensive article cited, he fully set forth its ecological characters and catalogued all available herbarium specimens. These showed its range to extend westward from Connecticut to northern Ohio, Wisconsin, and northern Minnesota. The type locality in Minnesota, and the most westerly station then known, was Mud River, Vermillion Lake, Saint Louis County, lying in the extreme northeastern part of the state, about 75 miles north of Duluth. ROBINSON and FERNALD[4] extended the range to Alberta, while the writer[5] has reported the species from Teton County, Montana. SCHNEIDER extends its range eastward to Newfoundland, north to the eastern shore of James Bay and the Severn River in Keewatin, and west to Edmonton, Banff, and Crow's Nest Lake in Alberta. The specimens cited later extend the range southwestward to Pembina and Rolette counties in North Dakota, and to Flathead County in extreme northwestern Montana. Both the Montana specimens come from the east side of the Continental Divide. Teton County lies on the plains at the eastern base of the Rocky Mountains, at an average elevation of about 4000 ft. Choteau is on the Teton River, which arises in the high mountains, but here flows eastward through the plains to the Missouri River. The localities in North Dakota are a southward extension of the distribution in Manitoba, while those in Montana obviously represent a similar extension of its distribution in the mountains of Alberta. It is quite possible that further search will extend the range both north and south in the Rocky Mountains. The Kennicott specimen from Slave River extends the range far to the north of Edmonton, into Athabasca or Mackenzie.

MONTANA.—Choteau County, Choteau, on Teton River, about 4000 ft. elevation, lat. 112°10′ W., *Griffiths* and *Lange*, August 22, 1900 (B); Flathead County, 3–4 ft. high in open marsh along Swiftcurrent Creek below Lake McDermott, alt. about 1350 m., *P. C. Standley* 16053, July 20, 1919 (B, N).

[4] ROBINSON and FERNALD, in GRAY, New Man. Bot. 322. 1908.

[5] BALL, C. R., in COULTER and NELSON, New Man. Rocky Mt. Bot. 130. 1909.

ALBERTA.—Crow Nest Lake, Rocky Mountains, *J. Macoun* 39 (Geological Survey Canada 94,440), August 8, 1897 (B); Rocky Mountains Park, Banff, low ground near the village, alt. 4500 ft., *W. C. McCalla* 2252, shrub 6 ft. tall, June 19, 1899 (N); vicinity of Banff, *N. B. Sanson* 304, July 14; 307, 309, 315A, 2167, July 15; 2173, June 27, 1911 (B); Calgary, *J. Macoun* 16 (Geological Survey Canada 94, 336), June 5, 1897 (B); Grattan Creek, near Battle River, *Macoun* and *Herriot* (Geological Survey Canada 70,252), August 17, 1906 (B).

ATHABASCA or MACKENZIE.—Slave River, *R. Kennicott*, July 1860 (N).

MANITOBA.—Bog north of Carberry, *Macoun* and *Herriot* (Geological Survey Canada 70,262), June 11, 1906 (B); near Sidney, *Macoun* and *Herriot* (Geological Survey Canada 70,263), June 12, 1906 (B) (70,264), June 13, 1906 (B).

NORTH DAKOTA.—Rolette County, Turtle Mountains, woods around Upsilon Lake (Fish Lake), *D. C. Mabbott* 464, September 7, 1917 (B); Pembina County, Walhalla, *L. R. Waldron* 1666, August 16, 1902 (B, ND).

ANDERSON in 1867 published *S. arguta** *S. pallescens hirtisquama*, based on a specimen collected by BOURGEAU at Lake Winnipeg and having short aments on short peduncles, scales densely white pilose except at tips, and narrow, sharply serrate leaves. Throughout its range *S. serissima* has short aments and pilose scales, but not narrow and sharply serrate leaves. The three Manitoba specimens cited do have such leaves, and it is quite possible that they represent this form. The leaves are not quite fully developed, and it seems hardly desirable to designate them as belonging to it without more and older material. On no. 70264 the under surfaces of the leaves show scarcely any traces of glaucescence. The leaves of all three are discolored in drying, however, which tends to obscure this character.

On flowering specimens from Manitoba (*Macoun* and *Herriot* 70262) and Alberta (*Sanson* 304, 309, 2167), a peculiar appearance has been observed. The capsules, nearly or quite full sized, but not mature, are minutely roughened or papillate, and the surface, viewed by reflected light, has a striking and deceptive resemblance to a fine lustrous puberulence.

SALIX LASIANDRA Bentham.—*S. lasiandra* Benth., Pl. Hartweg, 335. 1857.—*S. speciosa* Nutt., N. A. Sylva. **1**: 58. *pl.* 17. 1843. not HOST, 1828, or HOOKER and ARNOTT, 1832.—*S. arguta lasiandra* Anderss. Svensk. Vetensk. Akad. Handl **6**: 33. 1867 (Monog. Sal.).

—*S. lasiandra Lyallii* Sargent, Gard. and For. **8**:463. 1895.—*S. Lyallii* (Sarg.) Heller, Bull. Torr. Bot. Club **25**:580. 1898.

This beautiful species was described by BENTHAM from a staminate specimen, no. 1954, collected by HARTWEG on the Sacramento River in California. The cotype in the Gray Herbarium is a twig about 12 in. long, not fully in anthesis. The expanding leaves are only 2–4 cm. long and 5–9 mm. wide. The aments are 4 cm. long by 5–9 mm. wide.

The species had previously (1843) been described by NUTTALL from specimens observed abundantly on the Oregon and Wahlamet (Columbia and Willamette) rivers, and occasionally as far east as the Blue Mountains and the Boiseé (Snake) River.

It is a curious coincidence that FENDLER'S no. 816, collected near Santa Fe, New Mexico, and made by ANDERSSON the type of his *S. Fendleriana*, also is a staminate specimen with the aments not yet fully in anthesis and the leaves just unfolding. SCHNEIDER regards this specimen also as representing the true *S. lasiandra* rather than the green-leaved *S. caudata*, because, as he states, in some of the cotype specimens he has examined the leaves are more fully developed and show the glaucous under surface. Two specimens of this number in the National Herbarium are not sufficiently developed to show this.

The range of this species has been discussed recently by SCHNEIDER (Jour. Arnold Arb. **1**:17. 1919). Its distribution in Colorado and New Mexico, the southeasternmost extension of its range, is so restricted, and in a way so separated from the remainder, that the specimens known from these two states are listed below, in order to stimulate the interest of botanists.

COLORADO.—Montrose County, Cimarron, Gunnison River, alt. 6900 ft., *C. F. Baker* 141, June 15, 1901 (N); San Miguel County, Norwood Hill, river banks, alt. 7000 ft., *E. P. Walker* 453, August 11, 1912 (N); Archuleta County, Piedra (creek), *E. O. Wooton* 2718, August 12, 1904 (N, NM).

NEW MEXICO.—Rio Arribo County, Nutritas Creek below Tierra Amarilla, alt. 2250 m., *W. W. Eggleston* 6636, April 18–May 25, 1911 (N); meadows, vicinity of Chama, alt. 2380–2550 m., *P. C. Standley* 6645, July 9, 1911 (N); Sante Fe County, Sante Fe Canyon, 9 miles east of Sante Fe, alt. 8000 ft., *A. A.* and *E. G. Heller* 3637, June 2, 1897 (N); Sante Fe Creek, 4 miles east of Sante Fe, alt. 7500 ft., *A. A.* and *E. G. Heller* 3719, June 27,

1897 (N); McKinley County, north of Ramah, *E. O. Wooton*, July 25, 1906 (NM); Socorro County, Mogollon Mountains, middle fork of Gila River, alt. about 7000 ft., *E. O. Wooton*, August 4, 1900 (N); west fork of Gila River, alt. 6800 ft., *Wooton*, August 6, 1900 (N, NM); northwest of Mogollon Mountains, Lower Plaza, Frisco, alt. 5800 ft., *Wooton*, July 25, 1900 (N, NM); Frisco River, near Frisco, alt. 5800 ft., *Wooton*, July 25, 1900 (N).

SALIX LASIANDRA **Abramsi,** n. var.—Leaves narrowly lanceolate, 5–11 cm. long, 1–17 cm. wide, common sizes 6–7×1, 7–8×1–1.5, and 9–11×1.5 cm., margins shallowly serrulate to subentire; petioles short, 4–8–10 mm. long, thinly pubescent to glabrous, the glands of the distal upper surface small and inconspicuous or wanting; aments short, usually 2–3, sometimes 4 cm. long; capsules 5.5–7 mm. long; pedicels 1–1.5 mm. long.

This variety is named for Professor LEROY ABRAMS, of the Department of Botany of Stanford University, California, well known for his contributions to Pacific Coast botany and collector of the type specimen, his no. 4493, "near Sentinel Hotel, Yosemite Valley, Yosemite National Park, alt. 4000–4500 ft.," on June 23, 1911. It differs from the species chiefly in the smaller and narrower, less serrulate leaves, and the nearly eglandular petioles. It seems to be limited in its distribution to the Sierra Nevada of central eastern California, from Plumas County, south to Fresno County. Nearly all the specimens collected by DUDLEY in Nevada and El Dorado counties are immature and not identifiable with absolute certainty.

CALIFORNIA.—Sierra County, vicinity of Gold Lake, 1940 m., *W. W. Eggleston* 6263, 6265, August 28, 29, 1910 (N); Nevada County, lower end of Donner Lake, *A. A. Heller* 6879, July 8 (N, St.) 6943, July 16, 1903 (N, St.); vicinity of Donner Lake, *W. R. Dudley* 5007, 5008, June 12; 5018, 5026, 5027, 5049, June 14; Soda Springs station, *Dudley* 5138, June 15; flat land of the Yuba River opposite Cascade, *Dudley* 5149, 5150, June 15; by Truckee River, 1.5 miles below Truckee, *Dudley* 5155, June 17; Independence Lake, by outlet bridge, *Dudley* 5276, 5277, June 19 (all St.); Placer County, Monte Vista, Dutch Flat, *W. R. Dudley* (fol.), August 1909; El Dorado County, Glen Alpine Springs, *W. R. Dudley* 5660, June 1900 (St.); between Glen Alpine Spring and Camp Agazziz, *Dudley* 5664, June 27 (St.); Tallac House, Lake Tahoe shore, *Dudley* 5725, June 28, 1900 (St.); Glen Alpine, 6800 ft., *E. A. McGregor* 204, August 26, 1909 (St); Mariposa County, Mirror Lake, *W. R. Dudley*, June 12, 1894 (St), Yosemite National Park; near Sentinel Hotel, alt. 4000–4500 ft., *L. R. Abrams* 4493 (fem. type), June 23, 1911 (St); Merced Canyon, near Cascade Creek, 3500 ft., *Abrams* 4684, July 12, 1911 (St); Fresno County region of Sidney Creek, 5300 ft., *Hall* and *Chandler* 360, June 25–July 15 1900 (St).

SALIX CAUDATA **parvifolia,** n. var.—In the northern part of the range of *S. caudata* is found a form of lower stature and with

FIG. 1.—Portion of type specimen of *Salix caudata parvifolia* n. var. (nat. size)

shorter, narrower leaves (fig. 1). It occurs rather commonly and appears to be the dominant form in the mountains of northwestern

Montana and southern Alberta. While examination of a large number of specimens indicates that it passes gradually into the more typical form of the species, as do many other varieties, its recognition as a variety should help to a better understanding of the range of expression in *S. caudata*. Little is known of its height other than the notes given by STANDLEY, which indicate a lower stature than that of the species. The branchlets frequently are shorter and more divaricate; the leaves are very small, 5–8 cm. long, 7–12 mm. wide, seldom exceeding 1 cm. in width, common sizes being 6 cm. × 8 mm., 7 cm. × 9–10 mm., or on sterile shoots 8–10 cm. × 11–16 mm., strongly glandular-serrulate, as are the stipules also. The aments are 2–3 or 3.5 cm. long, rather lax; the scales 3–3.5 mm. long, linear-lanceolate, acute to truncate or toothed, and glabrate. The capsules are 6.5–8 mm. long.

The range of variety *parvifolia* is in the Rocky Mountains from Banff, Alberta, to the Yellowstone Park in Wyoming and the Wahsatch Mountains near Ogden, Utah, also in the mountains of western Idaho and eastern Oregon, and westward in Oregon to the eastern slope of the Cascades in Wasco County.

ALBERTA.—Rocky Mountains Park, *N. B. Sanson* 164 m., June 17, 1911 (B); 265, July 5, 1911 (B); 413, 414, August 21, 1911 (B); 2056, June 22, 1912 (B).

MONTANA.—Flathead County, Glacier National Park, 6–8 ft. high, boggy meadow, along Swiftcurrent Creek, below Lake McDermott, alt. about 1350 m., *P. C. Standley* 16865 (type) August 1, 1919 (N); thicket along lake, abundant, very slender, 6–12 ft. high, vicinity of Glacier Hotel ("Lewis's"), at head of Lake McDonald, alt. 900–1050 m., *Standley* 17906, August 22, 1919 (N); Deer Lodge or Powell counties, Deer Lodge Valley, mountain streams, 5000 ft. elevation, *J. W. Blankinship* 788, m. f., May 27, 1906 (N).

WYOMING.—Yellowstone National Park, Upper Fire Hole Basin, Yellowstone Lake, *J. M. Coulter*, Hayden Survey, July 1872 (N 253728, fr.); along Lamar Creek, *J. N. Rose* 406, fr., August 20, 1893 (N).

IDAHO.—Fremont County, along an irrigating ditch, St. Anthony, *Merrill* and *Wilcox* 899, fr., July 6, 1901 (B, N); Washington County, Weiser, alt. 2200 ft., *M. E. Jones* 6548, July 5, 1899 (N).

OREGON.—Union County, a small tree, bank of Catherine Creek, alt. 3500 ft., *W. C. Cusick* 2385, m. f. fr., May 30, June 28, 1900 (N); Grant County, Prairie City, alt. 1040 m., *W. W. Eggleston* 13700, September 5, 1916 (N); Wasco County, along streams in yellow pines, near head of Warm Springs River, alt. 3000 ft., *E. I. Applegate* 2777, September 7, 1898 (N).

UTAH.—Mountains near Ogden, Hayden's Expedition, 1872 (N, sheet 26198 in part, with *S. lutea* Nutt.).

SALIX LUCIDA Muhl.—I am at a loss to understand the discussion of the distribution of this species by SCHNEIDER. In his discussion of *S. lasiandra* (p. 16) he says:

> In 1867 ANDERSSON created two new species: *S. arguta* and *S. lancifolia*. To *S. arguta* he referred his *S. Fendleriana* of 1858 as a synonym, but only "p. p." Nevertheless he cited both specimens upon which he previously based his species, and added to them in the first place a specimen collected by BOURGEAU "ad fl. Saskatchavan, prope Carlton-house." This specimen (I have not yet seen the type in Herb. K.) probably belongs to *S. lucida*, and is identical with one of BOURGEAU'S specimens from the "Saskatchevan, 1859," preserved in Herb. G. Therefore the typical *S. argenta* of ANDERSSON consists of three different things, namely *S. lucida* (Bourgeau)—.

From this it would seem that SCHNEIDER thinks *S. lucida* is represented in Saskatchewan by two collections of BOURGEAU. Under *S. lucida* he states:

> There is likewise no proof that it occurs in Manitoba, Assiniboia, Saskatchewan, northeastern Alberta, Athabasca, and the Northwest Territories as far north as Great Bear Lake. Apparently *S. serissima* and *S. lasiandra* have been taken for *S. lucida*, of which the northeasternmost locality from where I have seen material is the Hill (or Hayes) River in Manitoba (*R. Bell*, August 1880, no. 24585, fr.; O.). But it seems very rare (or represented by *S. serissima*) in these regions and in western Ontario, becoming frequent to the east of Lake Huron in southeastern Ontario and southern Quebec.

The first two sentences are contradictory. One says that there is no proof of the occurrence of *S. lucida* in Manitoba, Saskatchewan, etc. The second states that the "northeasternmost" (northwesternmost?) locality from which *S. lucida* is known by him is in Manitoba, and he cites a specimen in the herbarium of the Canadian Geological Survey. Although the writer has seen no specimens of *S. lucida* from Manitoba, there is a strong probability that it occurs in that province. *S. serissima*, however, is much more common there, at least in a narrow-leaved form.

SALIX GOODDINGII Ball.—*S. Gooddingii* Ball, BOT. GAZ. **40:** 376. *pl. 12, figs. 2.* 1905; SCHNEIDER, BOT. GAZ. **65:** 12. 1918; SCHNEIDER, Jour. Arnold Arb. **1:** 9. 1919.—*S. nigra* of numerous authors, not MARSH.—*S. nigra vallicola* Dudley in ABRAMS, Fl. Los Angeles and vicinity. 100. 1904.—*S. vallicola* (Dudley) Britton, N. A. Trees 184. *fig. 141.* 1908.

This species was described in 1905 from a single collection of immature and somewhat parasitized pistillate specimens, and at that time placed in the section LONGIFOLIAE. Not long after describing it, I was indebted to Professor W. W. ROWLEE for calling my attention to the fact that the species belonged rather in the NIGRAE, and that GOODDING'S no. 719 represented the staminate plant.

FIG. 2.—*Salix Gooddingii* Ball: large trees on levee at border of Arizona Agricultural Experiment Substation, near Yuma, Arizona, showing form produced in open growth.

Such an error would scarcely have been made if mature specimens had been in hand. In the present instance the type specimen, with its puberulent to pubescent branchlets and tomentose capsules, constitutes so striking a departure from the characters so long associated with the species of section NIGRAE, and agrees superficially so well with those of far western members of the LONGIFOLIAE, that the deception was complete. Recently the writer has studied the numerous older collections of this species as well as some more recent material. Some interesting notes on habit, size, etc., have been obtained by Mrs. AGNES CHASE and the

writer (figs. 2–4). The rather abundant material and the fuller notes now permit a complete description of the plant, as follows:

Shrub 3 mm. tall, to tree 3–9 dm. in diameter and at least 12 and probably 15 m. in height; bark furrowed, gray; branchlets straight, slender, yellowish, glabrous to puberulent, more or less shining, seasonal twigs usually densely pubescent to subpilose; bud scales small, 2–4 mm. long, color and pubescence as in branchlets.

Leaves numerous; stipules 1–3 mm. long, or 8–10 mm. long on vigorous shoots, semiclordate to subreniform or sublunate, glandular-denticulate to dentate, often densely glandular on the upper (inner) surface also (see *Ball* 1821, 2069; *Chase* 5517); petioles 3–6 mm. long, yellowish, densely pubescent to glabrate; blades linear-lanceolate, usually somewhat falcate, 8–15 mm. wide, 6–10 cm. long, commonly 8 mm. by 8 cm., on new shoots up to 2.4 by 15 cm., usually acute at base, acuminate at apex, margins finely and shallowly glandular-denticulate with about 8 teeth per cm., green or yellowish green on both sides, often pubescent or puberulent until half grown, usually glabrous at maturity or the midrib beneath permanently pubescent; veins prominent above.

Aments coetaneous, numerous, solitary, terminating lateral leafy peduncles 2–4 cm. long, and bearing 3–6 small leaves; rachis densely pubescent to pilose; scales oblanceolate to lanceolate-oblong, or the staminate obovate, occasionally toothed or even lacerate at apex, 2.5–3 mm. long, yellow, more or less densely pilose, sometimes nearly glabrous on outer apical portion, deciduous; pistillate aments (originally described from immature parasitized specimens) 3–6 or 8 cm. long, 1.5–2 cm. wide, lax; capsules ovate-conic, 5.5–7 mm. long, roughened, thinly to densely pilose with gray hairs at anthesis, becoming glabrous at maturity; pedicels 2–3 mm. long, pilose, becoming glabrous; style less than 0.5 mm. long; stigmas divided, 0.3–0.5 mm. long; staminate aments 4–6 or 7 cm. long, 1–1.2 cm. wide; stamens 5–6, filaments pilose on lower third or half.

S. Gooddingii is found along streams and about springs from southwestern New Mexico to southern Nevada (Lincoln County), Baja California, and thence northward through the interior of California to Tehama County, in

FIG. 3.—*Salix Gooddingii* Ball, showing forms produced under conditions of previous over-crowding; near Yuma Experiment Farm of U.S. Department of Agriculture, in California, near Yuma, Arizona.

the vicinity of Red Bluff. It is most abundantly distributed in the valleys, having an elevation of only 0–200 ft., but ascends the foothills streams to 1500 ft. or more. The specimens listed later are referred to this species. The arrangement is from east and south to west and north. According to SCHNEIDER, this species is found as far east as the Rio Grande Valley in south central New Mexico and in the Davis Mountains of southwest Texas. The material from those districts is discussed later.

NEW MEXICO.—Grant County, Dog Spring, *E. A. Mearns* 183 (tree 25 ft. high, 1 ft. in diam.), May 29, 190 (3?) (N); Dog Spring, Dog Mountains, *Mearns* 2419, September 22, 1903 (N); tree 20 ft. high, Emory Spring, at foot of Emory Peak, *Mearns* 277, June 4, 1902, (N); near Kingston, in meadows, at 6600 ft. elevation, *O. B. Metcalfe* 969, 1904 (N); Mangas Springs, 18 miles northwest of Silver City, alt. 4770 ft., *Metcalfe*, April 26, 1903 (N); Gila, alt. 4200 ft., *E. A. Goldman* 1561, October 9, 1908 (N).

ARIZONA.—Graham County, Sierra Bonita Ranch, 23 miles north of Willcox, *R. A. Oakley*, 1904 (B); Duncan, *J. N. Rose* 11737, April 1908 (N); Cochise County, Ft. Huachuca, *Dr. Edward Palmer* 452, April 26–May 21, 1890 (N); *Dr. Patzky* (?), 1890 (N); *T. E. Wilcox* 63, 1894 (N); Chiricahua Mountains, Joe Smith's Ranch, alt. 5500 ft., *J. C. Blumer* 2306, November 22, 1906 (B); Bonita Canyon, alt. 6500 ft., *Blumer* 2309, November 4, 1906 (B); Santa Cruz County, Nogales, *I. Tidestrom*, March 28, 1908 (B): near Santa Cruz River, east of Nogales, *Tidestrom* 743, March 30, 1908 (B): Sonaita Creek, Patagonia, *F. M. Chamberlain* 5, April 2, 1904 (N); in creek bed at Patagonia, *Tidestrom* 814, April 10, 1908; Calabases, common in bottom lands, *Tidestrom* 870, April 21, 1908 (B), same locality, *Tidestrom* 886, April 24, 1908(B); Pima County, Canoa to Arabaca (Arivaca) *D. Griffiths* 3667, March 13–April 23, 1903 (N); Tucson, *Mearns* 178 (2658) November 21, 1893 (N); *J. J. Toumey*, April 13, May 20, 1894 (N); March, May 16, 1896 (N); *Myrtle Zuck*, May 16, 1896 (N); *G. R. Vasey* 266, March 1881 (N); *J. N. Rose* 11767, April 16, 1908 (N); *Rose, Standley*, and *Russell* 15192, April 27, 1910 (N); *Blumer* B 16, alt. 2400 ft., April 15, 1907 (B); Santa Cruz River, near Tucson, *Blumer* B 16a, May 10, 1907 (B.N,); Santa Catalina Mountains, alt. 3000 ft., *Blumer* B 17, April 25, 1907 (B.N,); Santa Rita Mountains, Andrade, *Griffiths* 4079, April 18, 1903 (B.N.); Pinal County, near Dudleyville, *Griffiths* 3666, March 13–April 23, 1903 (N); Yuma County, Yuma, State Experiment Substation, *C. R. Ball* 1740, 1741, June 15, 1911 (B,N); *Ball* 1901, May 26, 1915 (B,N); Mohave County, Topock, abundant along Colorado River, alt. 600 ft., *E. A. Goldman* 2970, September 27, 1917 (N); Beaverdam, alt. 1800 ft., *M. E. Jones* 5020, April 5, 1894 (N); Littlefield, near petrified springs, *I. Tidestrom* 9236, April 29, 1919 (B); at spring 8 miles above Pierce's Ferry, alt. 1700 ft., *Jones* 5077u, April 18, 1894 (N); locality unknown, Fremont's Expedition to California, no. 202 (A), 1845 (N), has "Utah" written on label, but "Ariz." added by same hand that added number and date; Beaver Creek, *B. E. Fernow*, August 1896 (N).

FIG. 4.—*Salix Gooddingii* Ball, showing character of bark on large trees, near those shown in fig. 2.

NEVADA.—Lincoln County, Muddy Creek (R) near Virgin River, *L. N. Goodding* 689, (*type*), May 2, 1902 (B, N); Rioville, Colorado River, *Goodding* 719, May 6, 1902 (B, N); along ditches, Bunkerville, *I. Tidestrom* 9202, May 27, 1919 (B); Nye County, Ash Meadows, *Coville* and *Funston* 2145, March 1891 (N), sub nom. *nigra venulosa.*

MEXICO.—Baja California, Seven Wells on Salton River, *E. A. Mearns*, 2869 (Internat. Boundary Commission), April 8, 1894 (N); *L. Schoenefeldt* 2877 (Internat. Boundary Commission) April 9, 1894 (N).

CALIFORNIA.—Mexican Boundary, Unlucky Lagoon, *L. Schoenefeldt* 2918, May 1, 1894 (N); Imperial County, Yuma (Fort Yuma Indian Reservation) pumphouse at ferry, *C. R. Ball* 1741, June 15, 1911 (B); Indian Reservation, *Agnes Chase* 5517, April 7, 1910 (B); Salton Basin, *S. B. Parish* 8092a, June 30, 1912 (B); San Diego County, Bernardo, San Dieguito River, *L. R. Abrams* 3371, May 2, 1903 (N); Pine Valley, *E. A. Mearns* 3977, August 12, 1894 (N); Orange County, Santa Ana River, near Orange, *L. R. Abrams* 3256 (type of *S. nigra vallicola* Dudley) April 16, 1903 (N); San Bernardino County, Colton, *M. E. Jones* 3195, April 28, 1882 (N); Fort Mojave, Mojave River, *J. G. Cooper*, March 25, 1861 (N, 319845); undated (N, 319846); Inyo County, on the old Mitchell Ranch, Resting Springs Valley, alt. 525 m., *Coville* and *Funston* 262, February 6, 1891 (N); Furnace Creek Ranch house, Death Valley, *Coville* and *Funston* 469, March 24, 1891 (N); Kern County, on the Tulare Plains, about 10 miles south of Bakersfield, alt. 400 m., *Coville* and *Funston* 1236, July 13, 1891 (N); Tulare County, Hanford, *Alice Eastwood* 3846, 3851, March 24, 1914 (N); Visalia, *Eastwood* 34, May 11, 1894 (N); Madera County, Fresno River, *J. W. Congdon*, June 21, 1903 (N); powerhouse no. 1, San Joaquin River, alt. 1000 ft., *E. G. Dudley* 5, November 1911 (B); San Joaquin County, large tree, 10–18 in. diam., in Tom Payne's or Paradise Cutoff, Tracy pike, about 10 m. south of Stockton, *C. R. Ball* 1929, June 1, 1915 (B, N). Amador County, Sutter Creek, Ione, *C. H. Merriam* 4, September 15, 1905 (letter) (N); South Jackson, 1500 ft., *Geo. Hansen* 198, July 3, 1892 (N); Sacramento County, Sacramento, *L. F. Ward* 89, October 1, 1895 (N); Sacramento Valley, Wilkes Exploring Expedition 1234 (N); Lake County, bank of Cache Creek, *H. N. Bolander* 2678 (N), 1863; Clear Lake (not certainly in Lake County), *J. Torrey* 490 (N), 1865; Yolo County, near Madison, *A. A. Heller* 5419, April 29, 1902 (N); Rumsey, *C. F. Baker* 2936, May 7, 1903 (N), Butte County, Biggs, near United States Experiment Farm, *C. R. Ball* 1820, 1821 (B, N), 1822, 1824 (B), August 15, 1913; same place, *Ball* 1939 June 4, 1915 (B, N); Chico, bank of Chico Creek, *Ball* 2069, June 15, 1916 (B); Tehama County, Red Bluff, *L. E. Smith*, 596, 599, 600, March 26, 1914 (N); 668, 669, May 8, 1914 (N); Shasta County, Reed Creek, *L. E. Smith* 610, March 30, 1914 (N).

In addition to this distribution, SCHNEIDER (BOT. GAZ. **65**:12–13. 1918; Jour. Arnold Arboretum **1**:9. 1919) credits *S. Gooddingii* with an eastern extension of range to central southern New Mexico and southwestern Texas

(not "northwestern," as SCHNEIDER states). The specimens so determined by him are listed later. Two chief districts are involved. The localities in Dona Ana County, New Mexico, and El Paso County, Texas, are in the Rio Grande Valley near El Paso, Texas. The Davis Mountains are some 100 miles to the southeast, forming part of the watershed between the Rio Grande and the Pecos rivers. I am by no means convinced that all of this material represents *S. Gooddingii* instead of a form of *S. nigra*.

NEW MEXICO.—Dona Ana County, on the White Sands, alt. 3700–4000 ft., *E. O. Wooton*, August 24, 1899 (N, 3 sheets, twigs brown).

TEXAS.—El Paso County, near El Paso, *G. R. Vasey*, March 1881 (N, 2 sheets); *Vasey* 267, April 1881 (N, 2 sheets); *V. Havard*, November 1881 (N); without locality, *Havard*, 1881 (N 264239); Mexican Boundary Survey, chiefly in the valley of the Rio Grande below Donana, *Parry*, *Bigelow*, *Wright*, and *Schott* 1357 (N); Jeff Davis County (probably), Fort Davis, *V. Havard*, April 1885 (N); Davis Mountains, *S. M. Tracy* 187, April 24, 1902 (N); Tom Green County, Knickerbocker Ranch, along Dove Creek, *Frank Tweedy*, May 1880 (N) (strongly suggests *S. nigra Lindheimerii* Schn.).

SALIX LAEVIGATA **araquipa** (Jepson), n. var.—*S. laevigata* forma *araquipa* Jepson, Fl. Calif. 339, 1909.—The original description by JEPSON reads as follows:

Forma *araquipa* Jepson, n. form. Small tree; one-year-old shoot with dense close tomentum; brown tuft of hairs on old wood at base of season's shoot very conspicuous; leaves reddish brown above; catkins long and dense. Arbor parva ramulis annotinis cum denso appresso tomento; valde manifestus caespes fusci pili basi horni ramuli in ligno vetere; folia rufo-fusca supra; amenta longa artaque.—Dry gulches, Araquipa Hills, Solano County, May 2–6, 1891, W. L. J.

The type came from "dry gulches, Araquipa Hills, Solano County (California), May 2–6, 1891, *W. L. Jepson*." This county lies northeast of San Francisco. I have not seen the type specimen, but an examination of the material in the National Herbarium, as well as that in my own herbarium, shows that this variety is found rather rarely in central California, but occurs commonly in the southern part of the state, comprised in Los Angeles, Orange, Riverside, San Bernardino, and San Diego counties. The vesture of the seasonal twigs, the buds, the petioles, and even the basal portion of the midrib, especially beneath, makes such a striking contrast with the glabrous and shining epidermis of the typical form that forma *araquipa* seems worthy of varietal rank. It should be noted, however, that the conspicuous tuft of brown hairs

at the base of the seasonal shoots is found on many specimens of which the shoots themselves are glabrous. The following specimens are referred to this variety:

CALIFORNIA.—Sonoma County, near Sonoma, *A. A. Heller* 5348, April 23, 1902 (N); San Bernardino County, San Bernardino, *G. R. Vasey* 265, February 1881 (N); *S. B.* and *W. F. Parish* 1204, 1881 (N); alt. 300 m., *J. B. Leiberg* 3243, 3244, both in part, April 4, 1898 (N); Los Angeles County, Rivera, *E. Braunton* 364, May 10, 1902 (N); Los Angeles River near Rivera, *L. R. Abrams* 3253, April 14, 1903 (N); San Francisquito Canyon, elevation 1500 ft., *W. M. Moore*, October 7, 1912 (B); Orange County, Santiago Canyon in Santa Ana Mountains, *V. Bailey* 1185, July 17, 1907 (N); Riverside County, Barranca, in mountains east of Pigeon Pass, *F. M. Reed* 2279, March 15, 1908 (N); San Diego County, Campo, by streams, *C. G. Pringle* 332, April 18, 1892 (N); Fall Brook, *M. E. Jones* 2870, March 25, 1882 (N); Jacumba Hot Springs, near Monument 233, *E. A. Mearns* 3245, May 20; 3322, May 28, 1894 (N); Warner's Hot Springs, *Alice Eastwood* 2589, April 9, 1913 (N).

ARIZONA.—Beaver Creek, *B. E. Fernow*, August 1896 (sub nom. *amygdaloides*) (N).

SALIX LONGIPES WARDII (Bebb) Schneider.—*S. nigra Wardii* Bebb, U.S. Nat. Mus. Bull. 22. 114–115. 1881.—*S. longipes Wardii* (Bebb) Schneider, BOT. GAZ. **65**:22. 1918.

So far as known, this species has not been reported heretofore from any station north of the Ohio River. In the autumn of 1918, a specimen collected on the banks of the Ohio, in Perry County, Indiana, was found in a collection of Indiana willows received for identification from CHARLES C. DEAM, State Forester of Indiana. On asking his interest in getting more material, he was kind enough to visit the spot again in 1920 and make another collection. Both specimens show only the characteristic foliage, but there can be no doubt of their identity.

INDIANA.—Perry County, low bank of Ohio River about 6 miles east of Cannelton, *Chas. C. Deam* 26749, September 24, 1918 (B,D); same place, a sprawling shrub growing in crevices of rock, the branches about 3 ft. tall, probably submerged during the winter, at least, *Deam* 33220, October 1, 1920 (B, D).

The recorded northern range of the species is from Washington, D.C., northwestward up the Potomac Valley to Alleghany County, Maryland, and westward in Upshur County, West Virginia (about lat. 39° N.), and Fayette County, Kentucky (about lat. 38° N.). Neither Upshur County nor Fayette

County is near the Ohio River, although the latter is in the same latitude as Perry County, Ohio, and less than 100 miles east of it.

SALIX AMYGDALOIDES Andersson.—This species is mentioned only to note extension of its range into two states excluded by SCHNEIDER, who in the main has set very accurate boundaries for its distribution. These states are Arizona and New Mexico. These specimens bear mature ovate-lanceolate leaves, and there can be no doubt of their identity, as those of *S. Wrightii* are linear-lanceolate and shorter-petioled.

ARIZONA.—Navajo Indian Reservation, Tunicha Mountains, 7000 ft., *E. A. Goldman* 2909, August 20, 1917 (N).

NEW MEXICO.—San Juan County, near Farmington, 1550–1650 m.; *P. C. Standley* 7047, July 19, 1911 (N); Navajo Indian Reservation, vicinity of Shiprock Agency, 1425 m. elevation, *Standley* 7867, August 11, 1911 (N).

These localities are in the extreme northeastern corner of Arizona and the extreme northwestern corner of New Mexico, respectively.

It may be worth noting also that the excellent survey of Indiana being made by CHAS. C. DEAM, State Forester, shows, by specimens I have seen, that *S. amygdaloides* occurs in fifteen counties in the northern third of the state (3–4 tiers of counties), and at two outposts, Henry and Marion counties in the central part of the state.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

POLYPODIUM VULGARE AS AN EPIPHYTE[1]

DUNCAN S. JOHNSON

(WITH THREE FIGURES)

While *Polypodium vulgare* is common on rocks, may often grow on the trunks of fallen trees, or sometimes even creep a few feet up living trunks, I have not been able to find a definite report of its being really epiphytic in habit in the United States. SCHIMPER,[2] the first general student of American epiphytes or air plants, says (1888, p. 131):

> In the North American forests the shade plants of the soil would not be able, because of lack of moisture, to grow on the bark of trees. Thus the so common *Polypodium vulgare* ascends to the trees in North America, just as little as it does in central or northern Europe.

Observations made at Cockeysville near Baltimore, Maryland, latitude 39° 30′ N, shows that this polypody can grow and fruit for years as a true epiphyte, high up on the erect branchless trunks of living trees. The ferns were not growing in an unusually moist region, as was true of the epiphytic individuals of it reported by CHRIST[3] (p. 325) as growing near a waterfall as Montreux, or in the damp forests of Portugal (see also SCHIMPER 1888, p. 31). On the contrary, the trees bearing this fern in Maryland were near the top of a northward facing cliff, more than 100 feet above a small stream, and at the western end of a ravine which is about 125 yards wide at this level. Two dozen or more plants of this fern were found growing in the deep furrowed bark of six different chestnut oaks (*Quercus Prinus*). The clumps of polypody were at various heights up to 20 feet or more above the ground. Clumps of all sizes were found, showing that they had started on the tree from prothallia, and had not arisen from rhizomes that had climbed upward from the soil. With one exception they were all on the north side (between N.N.E. and N.N.W.) of single erect trunks.

[1] Botanical contribution from the Johns Hopkins University, no. 70.

[2] SCHIMPER, A. F. W., Die epiphytische vegetation Amerikas. 1888.

[3] CHRIST, H., Geographie der Farne. 1910.

The exceptional case was that of a set of several clumps on a tree which had two trunks from a point about 5 feet above the ground.

FIG. 1.—East side of forked trunk of *Quercus Prinus* (between 2 feet and 6 feet above soil) showing several clumps of *Polypodium vulgare* established as epiphytes on the bark; cards 2×5 inches in size; ×⅛.

The two forks of the trunk stood almost in a north and south line, and the crotch between them in an east and west direction. The larger clump of polypody, which bore more than forty full grown leaves, grew just below the fork on the east side of the tree (fig. 1). At 6 inches and at 2 feet below this, on the same side, were two smaller tufts of this fern (fig. 1). Both the latter evidently profited from the collection of considerable water by the fork above, part of which water was directed down the shallow grooves of the bark in which these two clumps grew. This somewhat more abundant water supply, which is likewise more constant, probably explains the presence of these tufts on the east side of the tree, while all the other clumps of this polypody seen were confined to the north sides of the trees. The other five trees on which this fern was growing had trunks that were perfectly straight and without forks or any branches for many feet above the ferns (fig. 3). There was thus no collection of rain, as in the forked trunk, but each clump of polypody was dependent entirely on the portion of water that chanced to run down the particular furrow in which it grew. The fronds of the polypody on the unbranched trunks, although barely half as large as those on the forked trunk, were quite mature, and many of them bore spores. The more favorable growing conditions on the forked tree were indicated not only by the larger size of the polypody itself, but also by the richer growth of bryophytes and lichens, which were much more abundant below the fork than above it on this tree, or than on any of the erect trunks (fig. 2).

Aside from the smaller fronds of the epiphytic polypodies, they apparently were not different from those growing on the soil. In both the rhizome was largely covered by epiphytic liverworts and lichens and sometimes by more or less humus. The external character and internal structure of the rhizome and of the leaf, even to the thickness of the cuticle and of the mesophyll of the latter, were quite alike in plants of both habitats. The roots of both epiphytic and terrestrial plants were abundant, closely matted, and thickly beset with root hairs. Many of these root hairs had one or more fungous hyphae running lengthwise through them. These hyphae could often be seen entering at the tips of the root hairs. Whether they have the function of mycorhizal fungi has not been determined.

FOOD OF EPIPHYTES.—Not only the water, which was running over the bark of the supporting tree, but also the indispensable mineral foods dissolved in it, are absorbed by the roots of the epiphytic fern. In the locality under discussion, as well as in the wet tropical forests where epiphytes are most abundant, there can be but minute traces of mineral dust from the forest covered soil

FIG. 2.—Several clumps of polypody from near *x* in fig. 1, showing epiphytic lichens and liverworts, fruiting fronds of fern above, and young plants developed from prothallia below *x* at left; $\times\frac{3}{4}$.

blown by winds to the tree tops, to be washed down over the trunks by rains. It is evident, therefore, that the air plant is really dependent on the tree not only for support, in an advantageous position for light, but it must also rely on the tree to raise from the soil the food salts needed. In other words, the mineral-containing substances, resulting from the disintegration of bark, twigs, and leaves of the supporting tree (or perhaps a neighboring one), and

which are then washed down to the epiphyte, must first be carried above the epiphyte by the water vessels inside the tree. The epiphyte is to this extent dependent on a physiological process of the living host, the upward conduction of water, which involves a considerable expenditure of energy. The mineral food demands made upon the tree by the epiphyte are thus somewhat equivalent to those made by the "half-parasite" of its host. The chief difference is that the mistletoe exacts its quota of salts (and of water also) from within the living host, before they have been

FIG. 3.—Trunk of *Quercus Primus* bearing at *x* and *y* (north side of tree) two tufts of *Polypodium vulgare;* upper tuft 9 feet above soil, others above this are 18 or 20 feet from ground; $\times \frac{1}{15}$.

used by the host itself, while the air plant gets its salts from the surface of the tree after they have served their function within it. The water obtained by the epiphyte of course has never been inside the supporting tree. If the mistletoe is to be called a "water (and salts) parasite," the epiphyte is a "salts saprophyte"; that is, it secures its mineral food from the dead and no longer functional portion of the supporting plant.

ORIGIN OF TEMPERATE ZONE EPIPHYTES.—SCHIMPER (1888) announced the very important generalization that the vast majority of all vascular epiphytes are of tropical origin. Of extratropical epiphytes he believed that only the relatively few types found in

the rain forests of southern Chile and of New Zealand, with perhaps a couple of epiphyte ferns in Japan and southern Australia, are indigenous in origin. The other temperate zone epiphytes of the Old World, of South America, and according to SCHIMPER all epiphytes of temperate North America, have acquired this habit while in the tropical forest. SCHIMPER stated that it is the most xerophytic of the tropical epiphytes, those growing on the branches of the relatively dry roof of the forest, that have wandered out across the neighboring savannas and subtropical forest and onward sometimes 10 or 15 degrees beyond the tropics to populate with epiphytes the warmer and moister of the neighboring temperate forests. Because of the adaptation of these epiphytes to the dry conditions at the top of the forest, they have been able, in spite of the still more rigorous conditions encountered there, to colonize certain temperate forests. For the epiphyte that migrates from the tropics to the temperate zone, probably the most critical adverse condition encountered is not the occasional hot, dry summer, but the periods of low humidity during the generally wet winter season, when cold, dry, northwesterly winds prevail, during which the evaporation rate is high and water cannot be absorbed by the frozen roots. For example, the writer has noticed that tufts of *Tillandsia usneoides*, hung on a deciduous magnolia tree each year in May, thrive and grow rapidly during the summer, and even look fresh and green after several frosts in the autumn. They ultimately succumb, however, to the cold dry westerlies of winter, even of so moderate a winter as that of 1920–21. The precise measurement of the evaporating power of the air at these low temperatures, a factor of prime importance also to terrestrial plants, especially evergreen ones, must await the invention of a practicable frost-proof evaporimeter. Possibly the exposure of the epiphyte to sunlight, when the supporting tree is bare of leaves, is directly injurious also, although this seems hardly likely, since this same *Tillandsia* is abundant on deciduous trees only 200 miles south of Baltimore, where the winter sunlight would probably be at least as strong. The sunlight of course must work harm indirectly by increasing transpiration, which probably explains the usual restriction of polypody to the north sides of the trees.

The epiphytic ferns and seed plants of temperate North America, such as *Polypodium polypodioides*, *P. aureum*, *Vittaria lineata*, *Psilotum triquetrum* Sw. (=*P. nudum* [L.] Griseb.), *Tillandsia usneoides*, and *Epidendrum conopseum*, and the eighteen others named by SCHIMPER, have each a more or less widespread distribution in the American tropics, from whence they have probably migrated northward. The occurrence of *Polypodium vulgare* as an epiphyte in temperate North America, therefore, has a very interesting bearing on this question of the possible origin of extra-tropical epiphytes. For this fern, although distributed across the whole north temperate zone, in the New World from western Canada to Maine and south to Missouri and Georgia, and from Great Britain to Japan and southward into Northern Africa, is not known in the tropics, neither have fossils of it as yet been found there. We have no adequate evidence, therefore, that *Polypodium vulgare* acquired in the tropics the epiphytic habit which it assumes occasionally in Maryland, and more frequently in the damp forests of Portugal and Azores (SCHIMPER, 1888; CHRIST, 1910). The occurrence of this fern (or a closely similar polypody) in Cape Colony suggests that it may have crossed the equator by land, but of this there is no positive evidence, and this view seems negatived by the lack of fossils in equatorial Africa, and also by the absence of this polypody at the present day from the temperate highlands of the eastern tropics. From what is known of the habitats of *Polypodium vulgare* it seems most probable that this species is primarily a terrestrial plant of temperate forests. It probably entered North America from Eurasia via Alaska, and thence spread southward and eastward. It has acquired great hardiness while living on dry rocky ledges, often with a very scant water supply, and with no more soil than can collect in a few minute cracks of the rock. Thus this temperate zone polypody has come to be able to persist also in some shaded situations, on the very precarious supply of water and minerals to be found on the trunk of a rough barked tree. This is clearly true in spite of SCHIMPER'S somewhat too positive statement (1888, p. 152) that "in the less damp North American forests the first step, the migration of the terrestrial plants to the trees, is impossible, and herewith the origin

of an indigenous epiphytic association is excluded from the beginning." This *Polypodium* seems evidently an endemic epiphyte of the temperate zone, and not one imported with this habit already formed from the tropics. It might well be designated a facultative epiphyte. In its ability to live on various substrata it closely resembles dozens of species of ferns and seed plants of the tropical forests which can be found growing, now on soil, now on dry rocks, and again as epiphytes on tree trunks.

It might perhaps be suggested that more of our temperate zone plants should prove able to live on trees. As a matter of fact, however, few of our saxicolous vascular plants are really as hardy as this polypody, the thick-cuticled leaves of which are capable of rolling up in dry weather and so of lessening transpiration. The combination of these two features, uncommon in plants of this region but common in epiphytic ferns of the tropics, is probably an important one in enabling this fern, and likewise its relative *Polypodium polypodioides*, to adopt the epiphytic habit. The evergreen leaves of *Polypodium vulgare*, which are also characteristic of most, although not of all epiphytes, are probably of great importance to this plant of shady deciduous forests. They enable it to carry on an important share of its photosynthetic work on any mild days between October and May, when abundant light reaches it because the surrounding trees are bare of leaves. In other words, while growing on soil or rocks this fern has developed more of these xerophytic characters, which fit it for living as an epiphyte, than perhaps any other vascular plant of the northeastern United States. It seems at the present time to be an indigenous temperate-zone epiphyte in the making.

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CHROMOSOMES OF CONOCEPHALUM CONICUM

AMOS M. SHOWALTER

(WITH PLATES IV, V)

The discovery of visible chromosome differences between the sexes in many animals has led to a very wide acceptance of the hypothesis that sex in animals is determined by the presence or absence of certain chromosomes. This discovery has stimulated botanists also to search for sex determinants, and experimental work has apparently demonstrated that in *Sphaerocarpos* and *Thallocarpus* two of the four spores formed by the division of a spore mother cell produce male plants and the other two female plants. In several dioecious mosses also experimental results indicate that the sex potentialities are probably separated in the reduction divisions. As yet, however, a visible chromosome difference between the sexes has been found in only two species of plants, *Sphaerocarpos Donnellii* reported by ALLEN,[1] and *S. texanus* reported by Miss SCHACKE.[2]

The present study of the chromosomes of *Conocephalum conicum* (L.) Dum. was begun primarily for the purpose of determining whether or not there exists a visible difference between the chromosomes of the two sexes in this species. The results in regard to this question are totally negative, but the chromosome number is found to be nine instead of eight, as reported by previous workers for the gametophytes of this species.

The material used in the greater part of the study was grown in the greenhouse in two separate cultures of male and female plants respectively. These cultures were started with thalli bearing old gametophores of the previous season's growth, collected

[1] ALLEN, C. E., A chromosome difference correlated with sex difference in *Sphaerocarpos*. Science, N. S. **46**:466–467. 1917.

———, The basis of sex inheritance in *Sphaerocarpos*. Proc. Amer. Phil. Soc. **58**:289–316. 1919.

[2] SCHACKE, M. A., A chromosome difference between the sexes of *Sphaerocarpos texanus*. Science, N. S. **49**:218. 1919.

November 8, 1919, in Parfrey's Glen near Merrimac, Wisconsin. The archegoniophores were removed to prevent the possibility of the development of male sporelings from the sporogones, which latter were well developed at that time. The plants grew rapidly, and apical tips of the plants of the two sexes were fixed at several different times during the latter half of December. A few almost mature antheridiophores fixed in the field and imbedded in paraffin were obtained from Dr. W. N. STEIL. In addition to this material Dr. ALFRED GUNDERSEN and Professor A. F. BLAKESLEE generously supplied living plants from stock received from Copenhagen, and Professor A. J. EAMES sent plants from Cascadilla Ravine, Ithaca, New York. Comparative studies were made on these plants, but all figures shown were drawn from the Wisconsin material.

Flemming's medium solution with 4 per cent of urea added was used in fixing. Paraffin sections 4–6 μ thick in the case of the apical tips and 3 μ thick in the case of the antherids were stained with Flemming's triple combination. The sections on a few slides were restained in Heidenhain's haematoxylin, but gave results less satisfactory than those obtained with the triple stain.

The resting nuclei and stages in the formation of the spirem were not examined critically in this study. Numerous nuclei in spirem stages and in equatorial plate stages were found in these preparations, but very few cases of spirem segmented into chromosomes not yet drawn into the equatorial zone of the spindle were seen. Evidently, as observed by FARMER,[3] the transition from the unsegmented spirem stage to the equatorial plate stage is very rapid, if indeed the migration toward the equatorial region does not begin before the segmentation of the spirem, as evidenced by the frequently observed tendency of the chromosomes to lie end to end in the equatorial plate (figs. 2, 3, 9). The limited number of observations, however, does not justify any conclusion on this point.

Judging by the large number of nuclei in the equatorial plate stage, a considerable pause in the movement of the chromosomes occurs at this point, which also coincides with FARMER'S observations. The longitudinal splitting of the chromosomes does not

[3] FARMER, J. B., On spore formation and nuclear division in the Hepaticae. Ann. Botany **9**:469–523. 1895.

become apparent until very late; in fact, it is perceptible only when the separation of the daughter chromosomes has actually begun (figs. 13–15). The chromosomes are in the form of bent, crinkly rods of varying lengths (figs. 1–11). The crinkliness is less apparent in the late metaphases and in the anaphases when the chromosomes are drawn out into smooth rods (figs. 12, 16, 19). As observed by ESCOYEZ,[4] they occupy a very definite plane in the equatorial plate stage; in polar view they are easily counted at this time, but in lateral view they appear as tangled masses (figs. 8, 14, 15). Only one case (fig. 11) was found of an equatorial plate stage in which the individual chromosomes could be traced with any degree of certainty in a lateral view, and a very few such cases in anaphases (figs. 12, 16, 19). The chromosome number is plainly nine in either sex (figs. 1, 2, 4, 9, and 18 female; figs. 3, 5, 6, 8, and 10 male), one of the chromosomes being very small. This small chromosome shows no constant difference in behavior from the other chromosomes, either as to its position on the spindle or in its time of division. In one case (fig. 15) it was found to have been divided earlier than the other chromosomes, and in another case it was found undivided in the equator of the spindles when the other chromosomes were in anaphase (fig. 19). Metaphases and anaphases in which the individual chromosomes are distinguishable are very rarely found, but if the small chromosome constantly led the way in division, as it appears to do in fig. 15, or if it constantly lagged, as seems to be the case in fig. 19, it should usually be visible in the metaphases and anaphases, even though the other chromosomes are not distinguishable one from another. Apparently the small chromosome ordinarily divides at about the same time as the other chromosomes, and in lateral view is distinguishable from them only in rare cases (figs. 12, 15, 16).

In cells of the apical tip of the thallus (of either sex) in which the chromosomes are commonly spread out so as to make accurate counts possible, the small chromosome is visible in about 80 per cent of the cases counted; but in the antherid, where the cells are much smaller and the chromosomes generally more closely grouped,

[4] ESCOYEZ, E., Blepharoplaste et centrosome dans le *Marchantia polymorpha*. La Cellule **24**:247–256. 1907.

it is visible in a much smaller percentage of the cases. Considering the size of this chromosome, it is to be expected that in some cases it should be obscured from vision by the other chromosomes (figs. 7, 8, 14, 17).

FARMER, BOLLETER,[5] ESCOYEZ, and WOODBURN[6] report eight chromosomes in the haploid nucleus, and in my preliminary note[7] I suggested the possibility of a variation as to chromosome number in this species. More recent studies in plants from Ithaca and from Copenhagen make it seem quite certain that the same number of chromosomes is to be found in the plants of this species in those regions. It seems probable, therefore, that these investigators have overlooked the small chromosome, a thing which might easily have happened, especially since they were interested primarily in other phenomena.

A comparison of the chromosomes of one sex with those of the other shows no perceptible difference, either in the number or size relations, as may be seen by comparing figs. 1, 2, 4, 7, 9, and 18 (female) with figs. 3, 5, 6, 8, and 10 (male). Although this condition of like chromosomes in the two sexes in *Conocephalum* is not an evidence against the sex chromosome basis of sex inheritance in the dioecious Bryophyta, it does show that the marked difference between the chromosomes of the two sexes in *Sphaerocarpos* is not a universal condition among these plants.

Summary

1. The chromosome number in the gametophyte of *Conocephalum conicum* (L.) Dum. is nine instead of eight as reported by previous investigators.

2. The chromosomes vary considerably in size, one being very much smaller than any of the other eight.

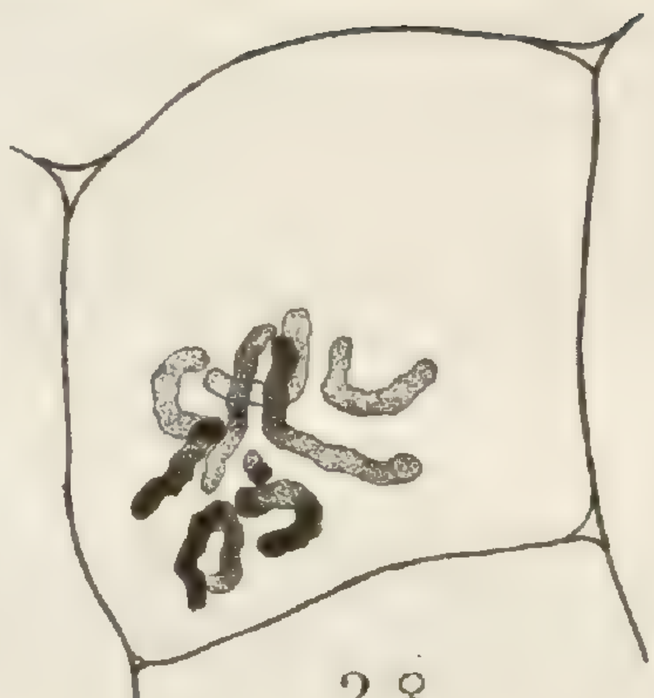
3. There is no perceptible difference between the chromosomes of the male and those of the female plant.

[5] BOLLETER, E., *Fegatella conica*, eine morphologisch-physiologische Monographie. Beih. Bot. Centralbl. **18**:327–408. 1905.

[6] WOODBURN, W. L., Spermatogenesis in certain Hepaticae. Ann. Botany **25**:299–313. 1911.

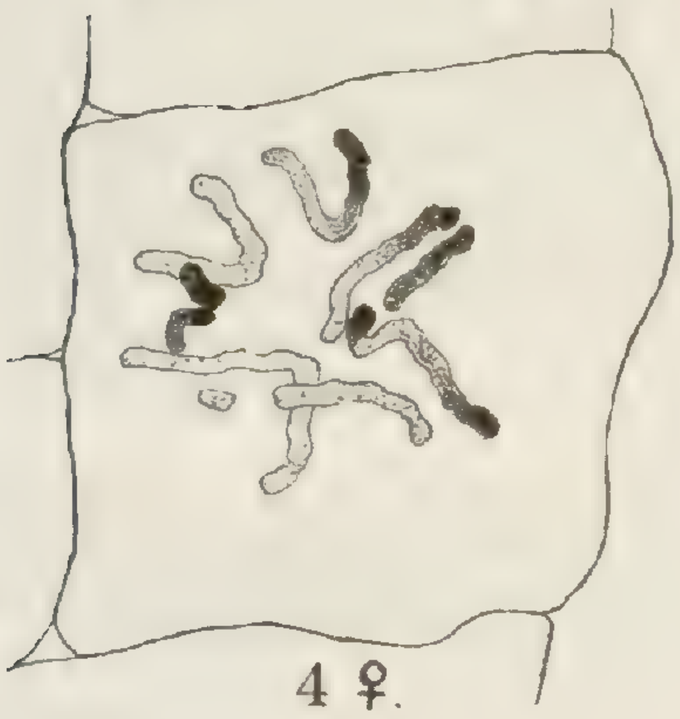
[7] SHOWALTER, A. M., The chromosomes of *Concephalum conicum*. Science, N. S. **53**:333. 1921.

1 ♀

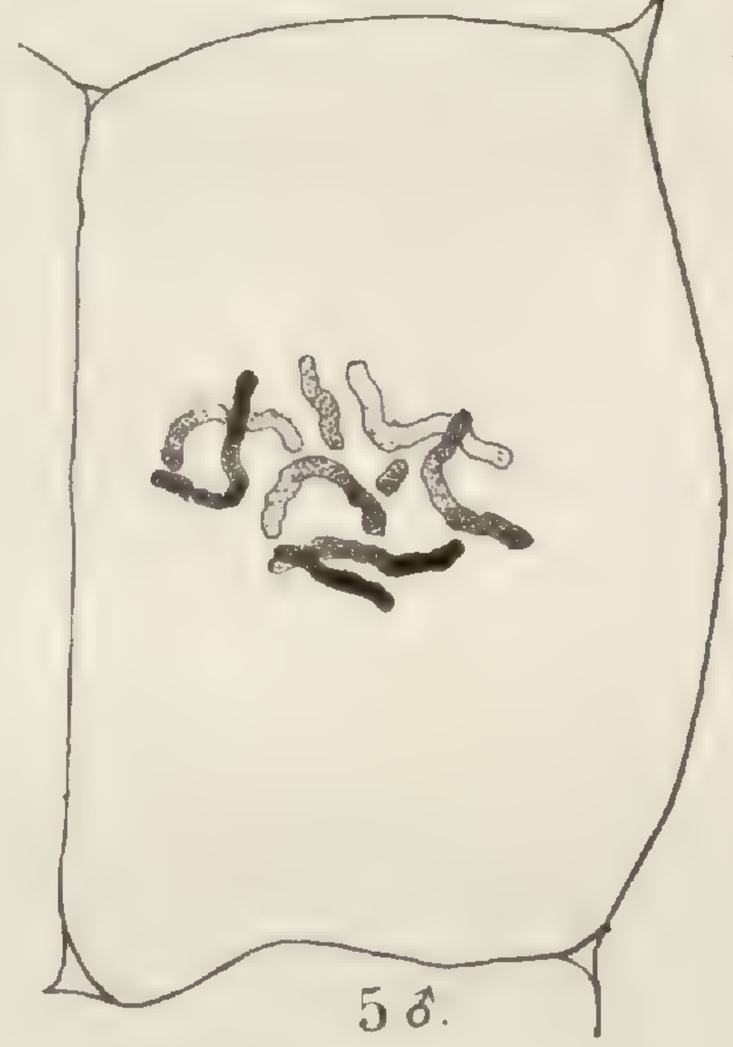


2 ♀.

3 ♂.

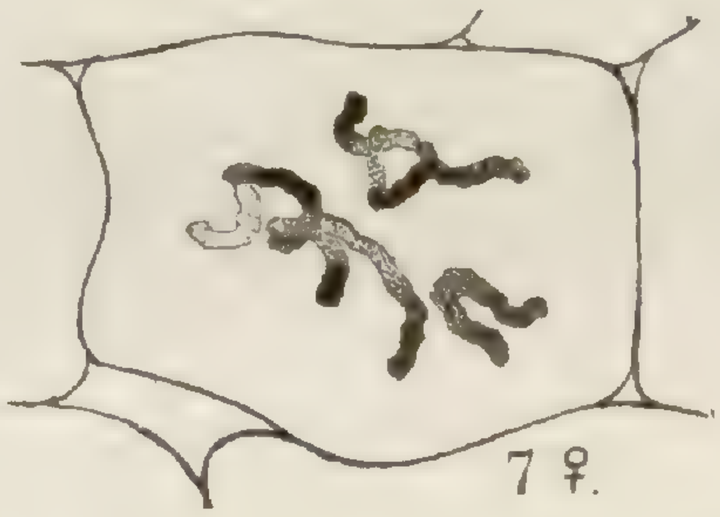


4 ♀.



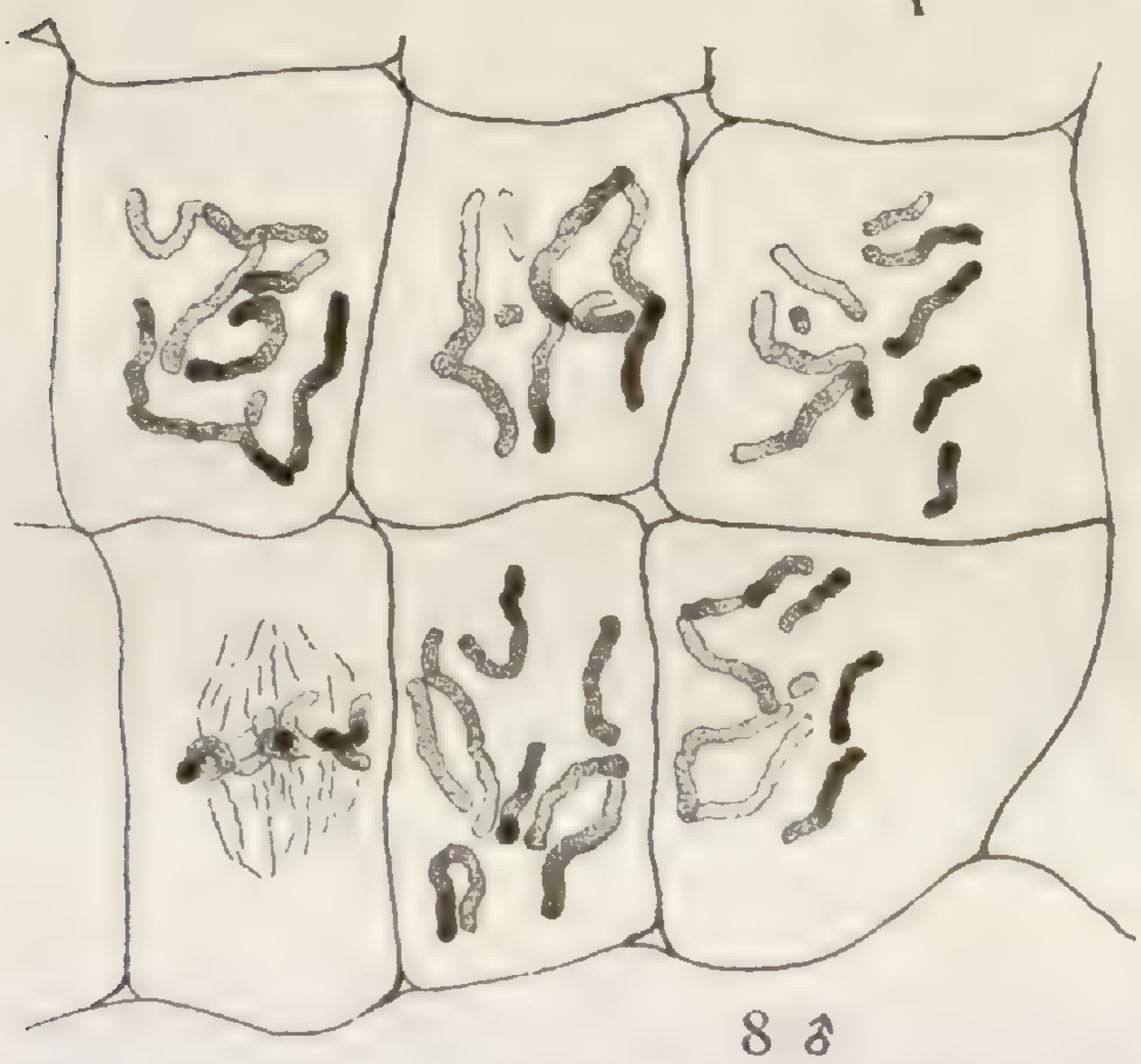
5 ♂.

6 ♂

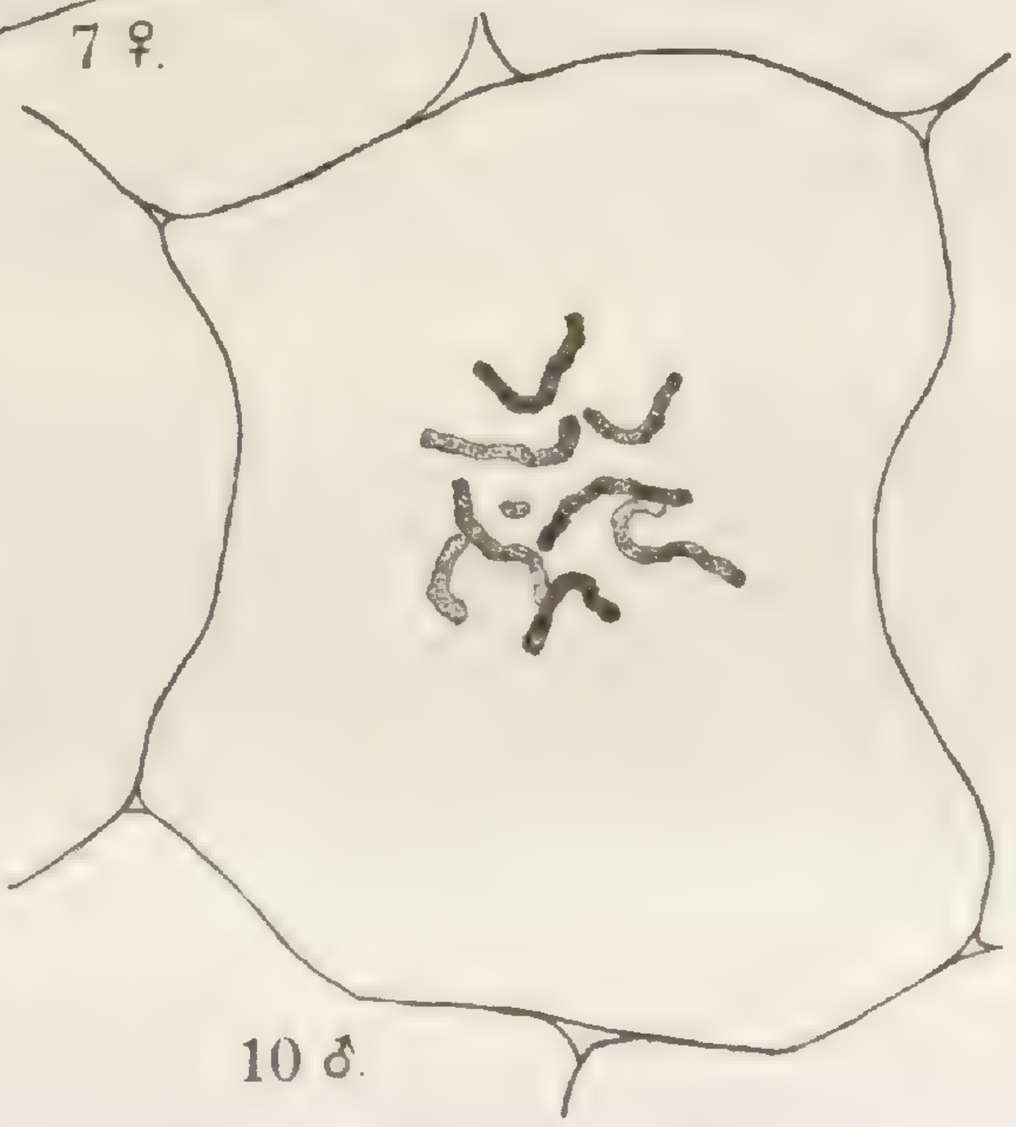


7 ♀.

9 ♀.



8 ♂

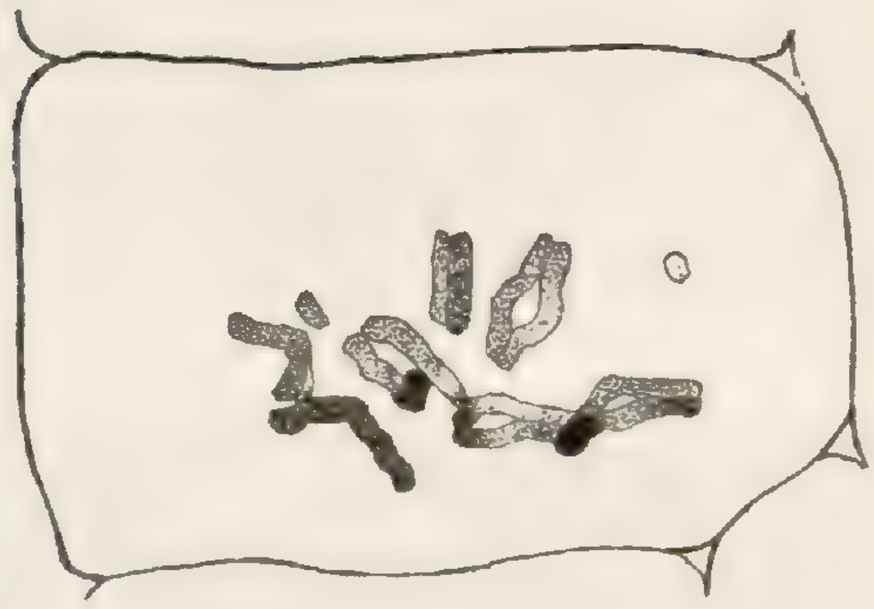


10 ♂.

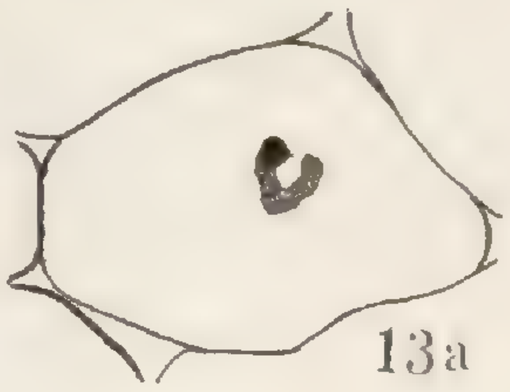
SHOWALTER on CONOCEPHALUM

11 ♀.

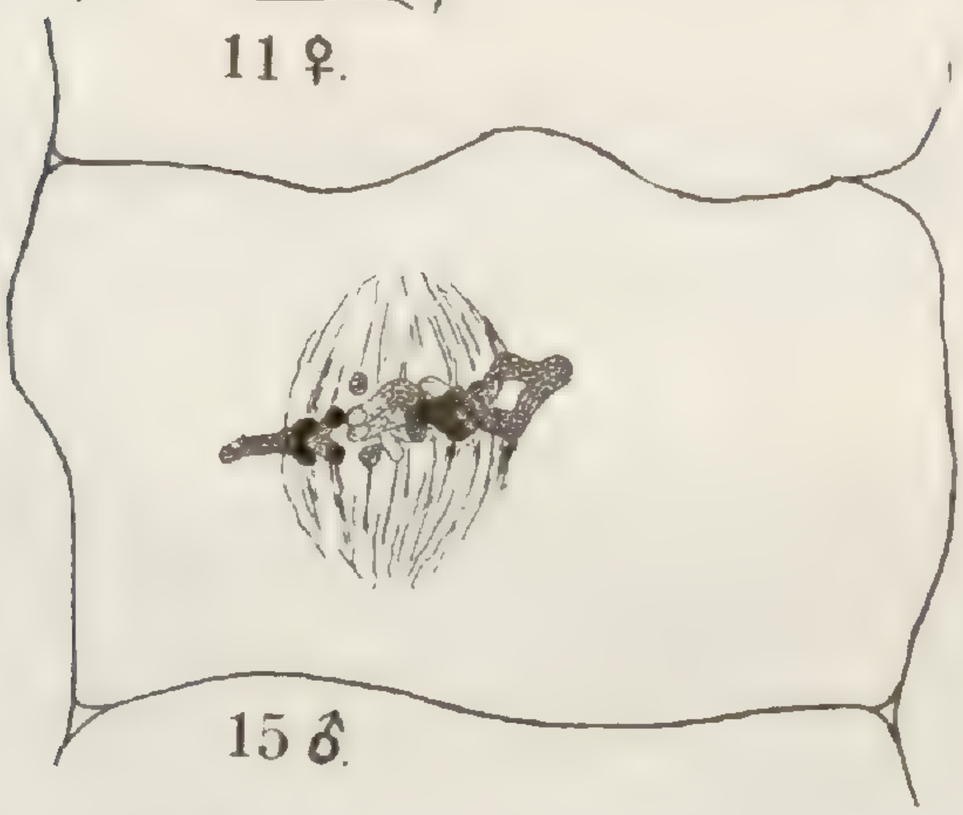
12 ♀.



13 ♀



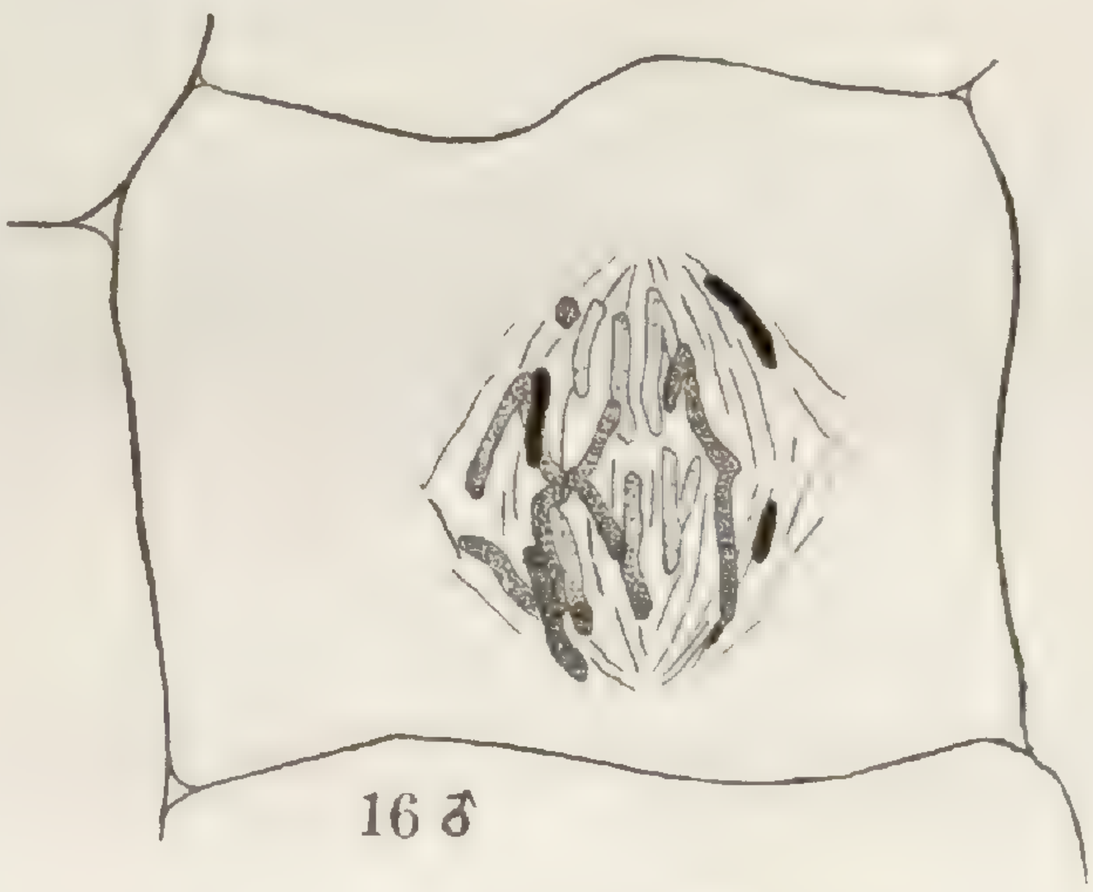
13a



15 ♂.

14 ♂.

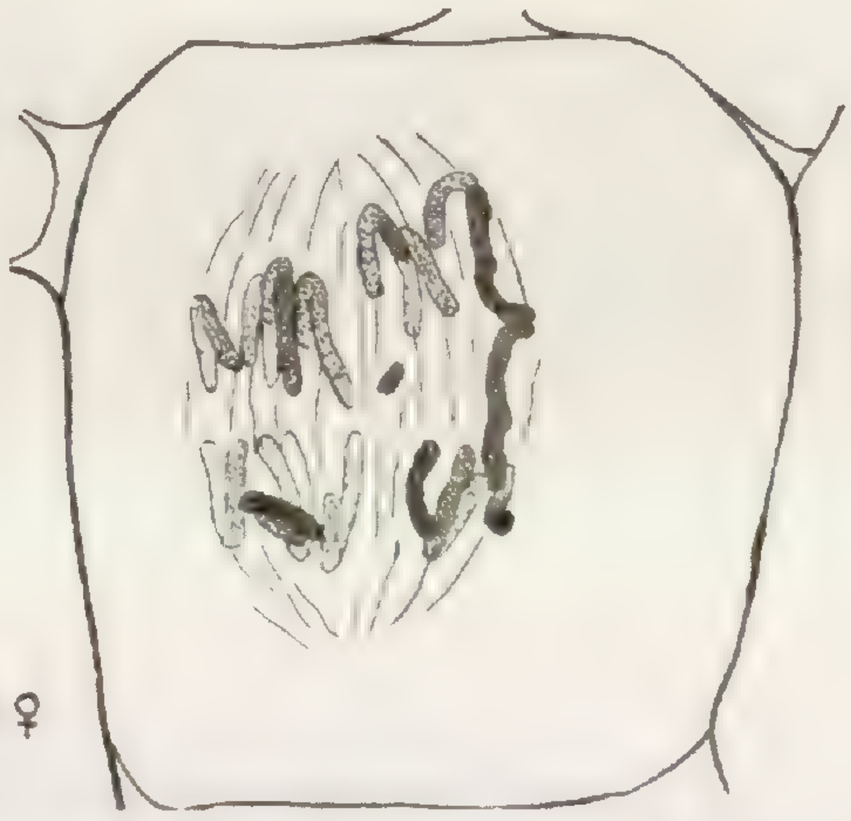
14a



16 ♂

17 ♀.

18 ♀



19 ♀

SHOWALTER on CONOCEPHALUM

4. The plants received from Ithaca and Copenhagen show the same number and size relations of the chromosomes as do the Wisconsin plants.

I wish to acknowledge my indebtedness and gratitude to Professor C. E. ALLEN, at whose suggestion and under whose direction this study has been made.

UNIVERSITY OF WISCONSIN

EXPLANATION OF PLATES IV, V

All drawings were made with the aid of a camera lucida at a magnification of about 3800, using a Zeiss 2 mm. apochromatic objective N. A. 1.40 and compensating ocular no. 18 with a tube length of 160 mm.

FIGS. 1–11.—Equatorial plate stages in polar view (some slightly oblique) except fig. 11 and one cell in fig. 8, which are in lateral view; symbol following number indicates sex in each case; fig. 1 from cell of dorsal surface layer near apical cell; figs. 3–6 from cells of ventral surface layer; in fig. 4 one chromosome at left upper focus cut in sectioning; figs. 7, 10, and 11 from cells of interior of thallus; fig. 8, group of six cells in antherid; fig. 9, cell of young scale.

FIG. 12.—Anaphase in cell of air chamber wall, small chromosome being visible in upper group only.

FIG. 13.—Early metaphase in cell of ventral surface layer, in slightly oblique (nearly polar) view; one chromosome, except tip of one end, in adjacent section shown in fig. 13*a*.

FIGS. 14, 14*a*.—Early metaphase in cell of interior of thallus, cut in sectioning; chromosomes not all distinctly recognizable.

FIG. 15.—Early metaphase in cell of scale, showing small chromosome already divided, other chromosomes not individually distinguishable.

FIG. 16.—Early anaphase in cell at juncture of scale and main body of thallus.

FIGS. 17, 18.—Anaphase daughter groups in successive sections of same cell, seventeen in polar view, eighteen in equatorial view; cell in floor of young air chamber.

FIG. 19.—Early anaphase in cell of ventral surface layer; small chromosome not yet divided.

PEACH YELLOWS AND LITTLE PEACH[1]

MEL. T. COOK

(WITH PLATES VI, VII)

Peach yellows and little peach are well known but poorly understood diseases, and have been the subject of study by many workers for a number of years. Although they have engaged the efforts of some of our most efficient workers, the causes are as yet unknown, the symptoms not well defined from similar symptoms due to some other common causes, and the methods of control are very unsatisfactory. Although the researches have been directed along many lines, very little attention has been given to the morphology of the organs of the infected plants as compared with the morphology of corresponding organs on healthy trees. The fact that a knowledge of the morphology is frequently a very important factor for physiological studies has led to the preparation of this paper, hoping that the accumulation of data along various lines may eventually assist some student to solve this problem.

The material used was taken from trees in an experimental orchard at Vineland, New Jersey, which was planted and managed by the Department of Horticulture of the New Jersey Agricultural Experimental Station. The trees were under constant observation, and there was no doubt as to their condition. The material was carefully collected during the early morning and mid-afternoon of a bright warm day in midsummer, when the conditions were exceptionally favorable for growth. Care was taken to select leaves of approximately the same age, and the same precaution was taken with the twigs. A great many sections were cut and a considerable number of drawings made, from which the figures shown in the plates were selected.

The studies were based on a comparison of the structure of corresponding parts, the relative amounts of starch in these organs morning and afternoon, and its relative location. The studies of

[1] Paper no. 29 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Pathology.

structure did not show any differences of importance, and will not receive further consideration at this time. The study, however, of the amount and location of the starch within the tissues of the leaves and green shoots gave some interesting data, and therefore the basis of the work is a comparative study of the results of photosynthesis and translocation of carbohydrates in healthy and diseased trees.

Before considering this phase of the work, the generally recognized symptoms of these diseases are indicated, since they must be referred to from time to time. The first symptom in both cases is an infolding along the midrib or rolling of the margins, accompanied by a pronounced backward curving from base to tip so as to give a sickle or crescent effect, and the development of a decidedly leathery texture which is very apparent to the touch. The second symptom for yellows is the development of enlarged, prematurely ripened fruits, which show a characteristic red spotting or blotching over the surface and through the flesh, especially prominent near the stone. The final stage in the yellows is the development of fascicled, willowy shoots. Very similar symptoms may be produced by partial or complete girdling of trunk or branch by winter injury at the collar, by borers, by label wires, or other factors. There is no doubt that many of the reported cases of peach yellows in the past were in reality cases in which the symptoms were produced by some of these causes. The first stage or leaf characters in little peach is similar to that of yellows, but is very likely to be more pronounced than in yellows. In the second stage the fruit is small and ripens later than in the normal healthy trees. There is no willowy growth as in the case of yellows. The symptoms just described are subject to many variations, dependent upon cultivation, care, and other factors. Yellow foliage may be due to many other causes, and is not necessarily a symptom of yellows. In fact, trees infected with this disease may be very green, especially if fed with a fertilizer high in nitrogen. Trees infected with yellows will sometimes persist for a number of years, but those infected with little peach usually die in a comparatively short time.

In a normal healthy plant the starch content is expected to be much greater in the afternoon than in the early morning, due to

the high photosynthetic activity during the day and the lack of photosynthesis and a very active translocation of starch during the hours of darkness. In this work a study of the sections of leaves from healthy trees removed early in the morning and in mid-afternoon was made for comparison with leaves from diseased trees which were collected at the same time. In the leaves from normal healthy trees it was found that there was very little or no starch in the leaves during the morning hours, and an abundance during the afternoon (figs. 1, 2.) This of course was to be expected, and indicated that the photosynthetic and translocation processes were normal and active on the day that the material was collected. In some instances a small amount of starch was found in the cells in the morning in the immediate vicinity of the veins (figs. 3, 4). This was no doubt due to incomplete translocation and may possibly indicate a slightly abnormal condition.

Leaves were collected from the varieties known as Stump, Hiley, and Chinese cling, which were affected with yellows. In those in which the disease was severe, the amount of starch in the sections from leaves cut in the morning was almost as great as the amount found in leaves cut in the afternoon, indicating little or no translocation of the carbohydrates (figs. 5, 6). The amount of starch, however, was not as great in either case as in the healthy Elberta (fig. 2) in the afternoon, but was greater than in the healthy Hiley (fig. 4). These differences in the amount of starch in the individual trees may be due to a difference in the physiological activities of the trees, and may possibly be accounted for by differences in variety, age, or other factors.

A morning section of a Chinese cling affected with yellows (fig. 7) compared with a morning section of a healthy tree of the same variety showed a much larger amount of starch in the leaf from the diseased tree than in the leaf from the healthy tree, indicating not only a reduced translocation of carbohydrates but also an accumulation of these products. There was very little difference, however, between the amount of carbohydrates found in the morning and afternoon sections from diseased trees (figs. 7, 8).

The little peach was studied on Elberta and Hiley. The amount of starch was practically the same in the sections from

leaves collected in the morning as in the afternoon (figs. 9, 10), but was less than in the trees affected with yellows (figs. 5, 6). In some other sections, however, the amount of carbohydrates in both morning and afternoon sections was greater than that shown in figs. 9 and 10. The starch in the sections from leaves cut in the morning was most abundant in the central part of the leaf (fig. 9), and may indicate some translocation. A morning section of leaf from Elberta infected with little peach (fig. 11) showed a large amount of starch, indicating that very little translocation of starch had taken place during the preceding night.

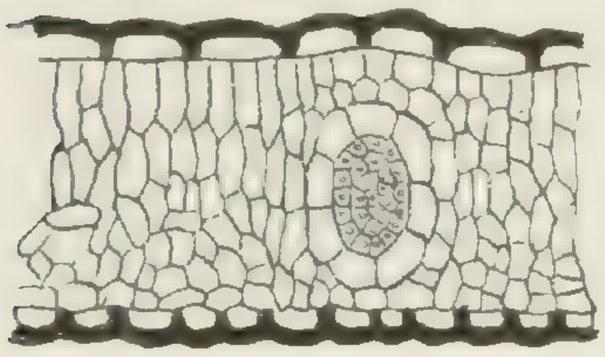
Pieces of new growth shoots were collected at the same time that the leaves and sections from Elberta, Stump, Hiley, and Chinese cling were studied. The results were practically the same throughout, but as the material from the Hiley was most abundant and most satisfactory it is used for this part of the discussion. A comparison of morning and afternoon sections of young shoots from a healthy tree shows a considerable amount of starch in the inner layers of cortex in the afternoon section (fig. 13) and very little in the morning section (fig. 12), indicating normal translocation of carbohydrates. These twigs were from the same tree as the leaves in figs. 3 and 4. The shoot from the tree affected with yellows (figs. 14, 15) was slightly older than the healthy shoot. The amount in the morning and afternoon was practically the same, indicating that there was little or no translocation of carbohydrates. These twigs were from the same tree as the leaves shown in figs. 5 and 6. The sections from the tree affected with little peach showed a slightly smaller amount of starch in the morning (fig. 16) than in the afternoon (fig. 17), which may possibly indicate that there was a small amount of translocation. These twigs were from the same tree as the leaves in figs. 9 and 10.

It will readily be seen that all these studies on both the leaves and the new growths indicate that the translocation of starch is partly or completely inhibited in the diseased trees, probably dependent upon the severity of the disease. This is indicated by the large amount of starch present in leaves and green twigs from diseased trees in the early morning, as compared with the relatively small amounts in leaves and green twigs from healthy trees at the

same hour. It is very generally recognized that girdling interferes with the translocation of carbohydrates, and as a result thereof bearing plants very frequently produce extra large fruits which usually ripen prematurely. The production of large premature fruits is also a characteristic symptom of yellows, and it therefore appears that the physiological behavior of a tree affected with yellows is the same or very similar (so far as photosynthesis and translocation of carbohydrates are concerned) as in a tree that has been girdled. In trees affected with little peach, however, the symptoms, so far as the fruit is concerned, are just the reverse, the fruit being somewhat smaller and ripening later than normally. This may possibly account for the fact that sections of twigs from trees affected with little peach showed some evidence of translocation of starch, while those from trees affected with yellows did not show any such evidence. These differences, however, may have been due to other causes, such as severity of infection, age of trees, or other factors.

The preceding discussion indicates that the translocation of starch is greatly reduced, possibly completely checked, in trees affected with either of these diseases; or that have been girdled and injured by label wires, bores, or at the collar as a result of freezing. In all cases the results are an accumulation of starch in the leaves, which may account for the leathery texture, but does not offer an explanation of the willowy growth of the final stage of the yellows. If the translocation of carbohydrates is reduced or prevented, however, it may have a secondary effect on the tree, resulting in the willowy growth.

Furthermore, the reduction or inhibition of the translocation of carbohydrates may also account for the enlarged premature fruit which is characteristic of trees affected with yellows, or that have been girdled, but it does not explain the undersized fruit and delayed ripening which is characteristic of trees affected with little peach. These facts indicate that some of the symptoms of these diseases are due to reduced or inhibited translocation of carbohydrates. The cause of this condition is a question that remains unanswered.



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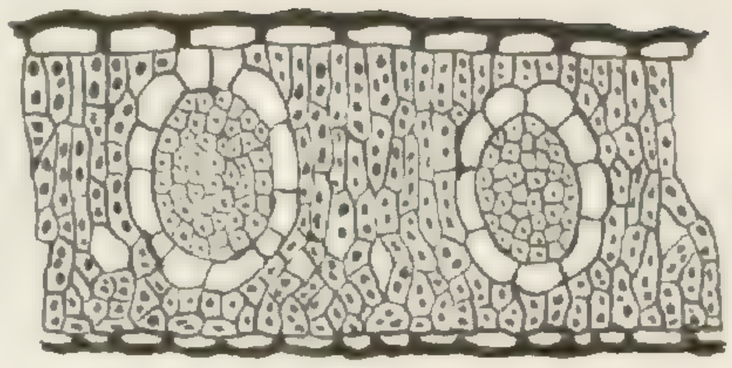

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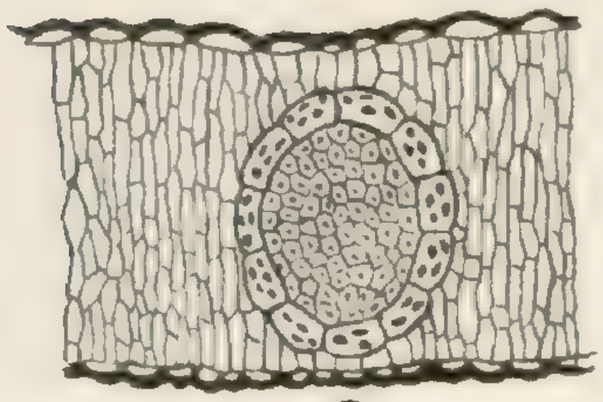

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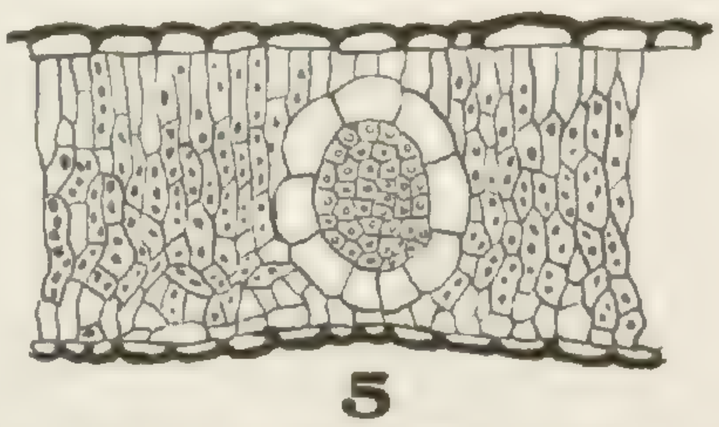

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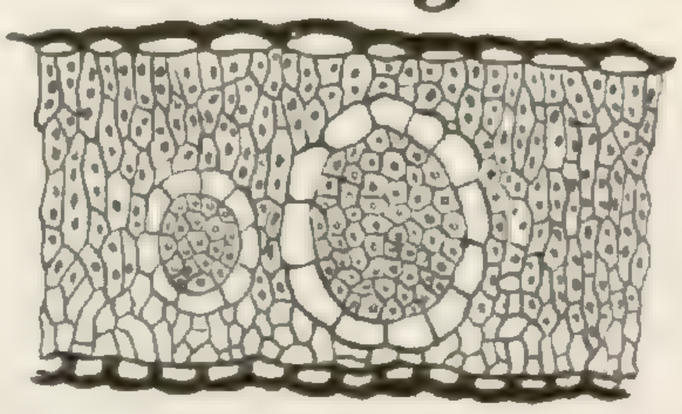

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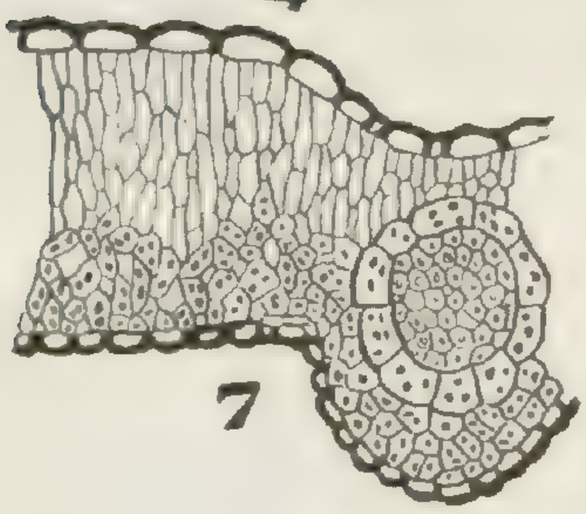

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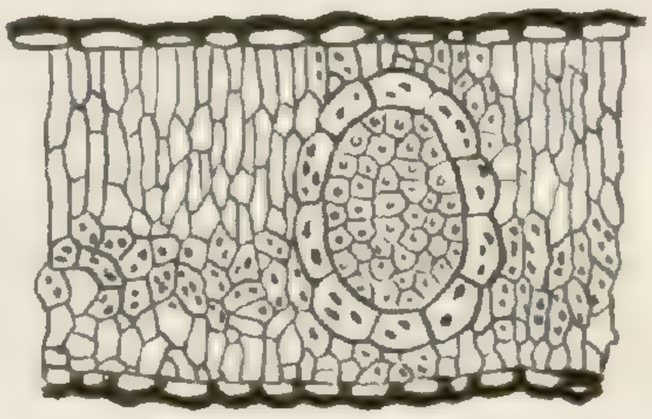

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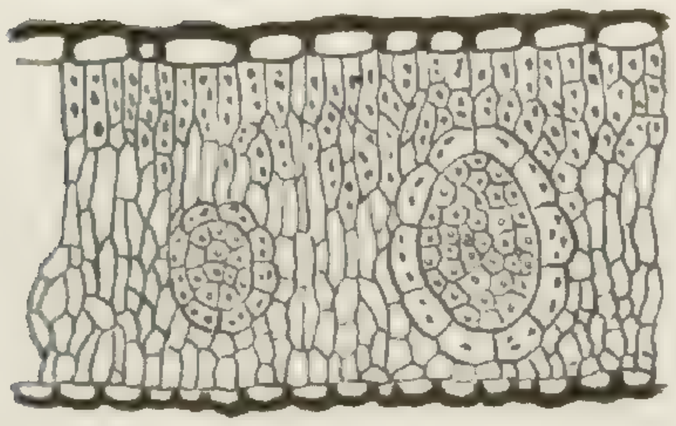

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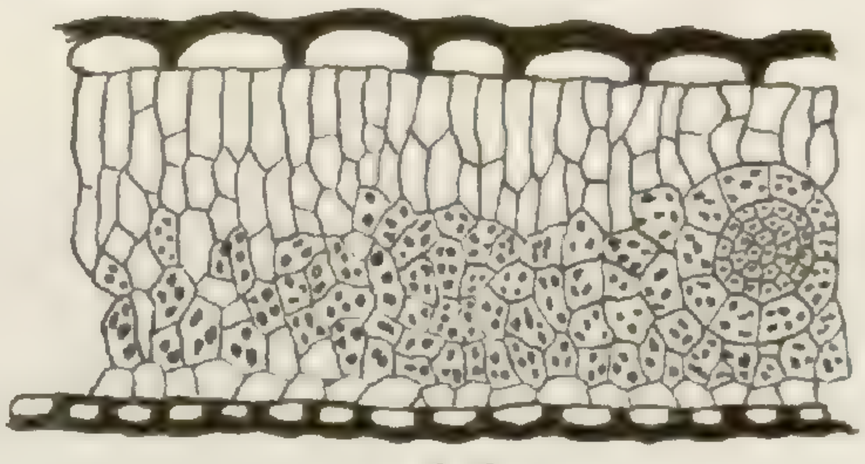

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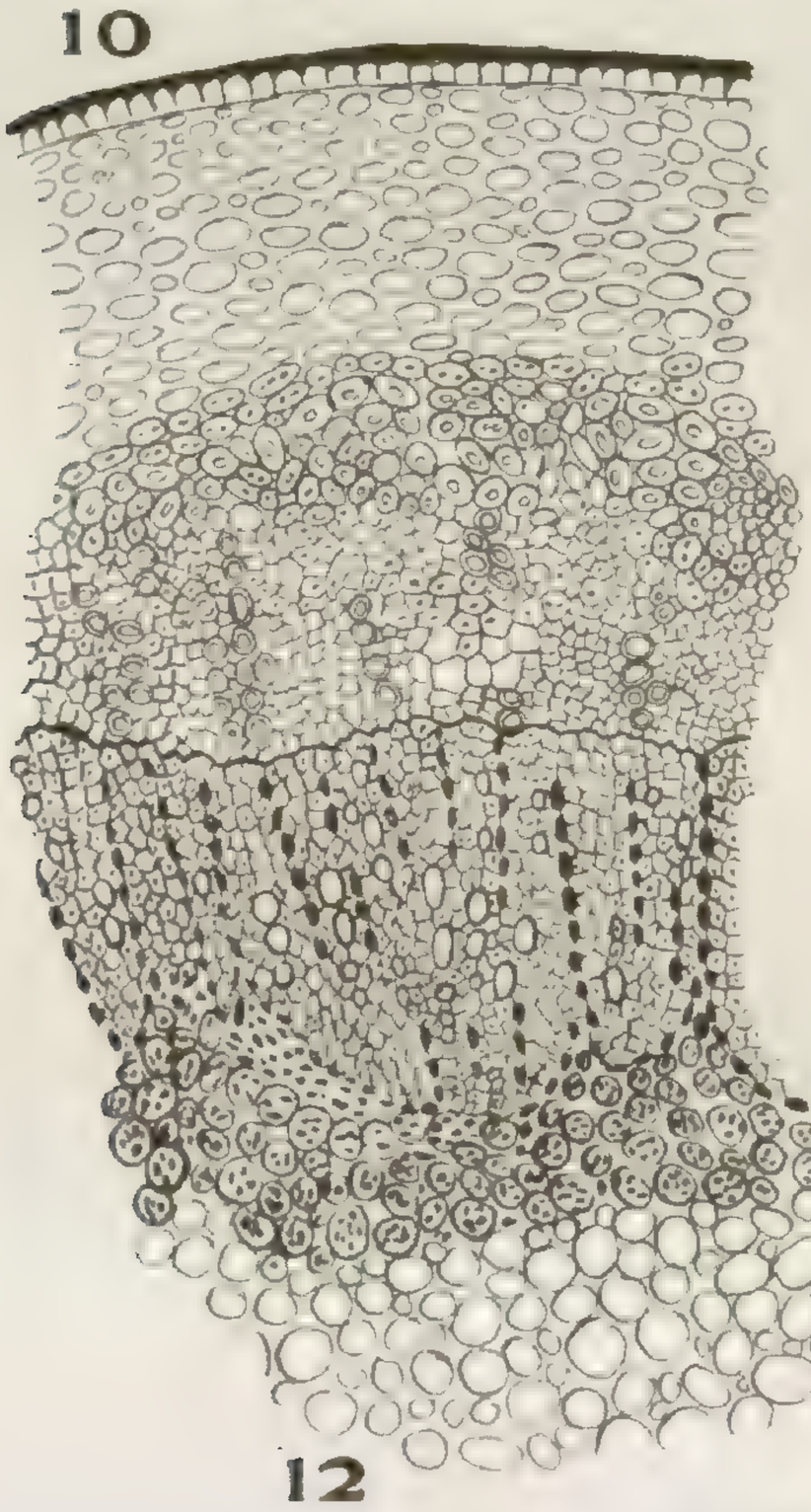

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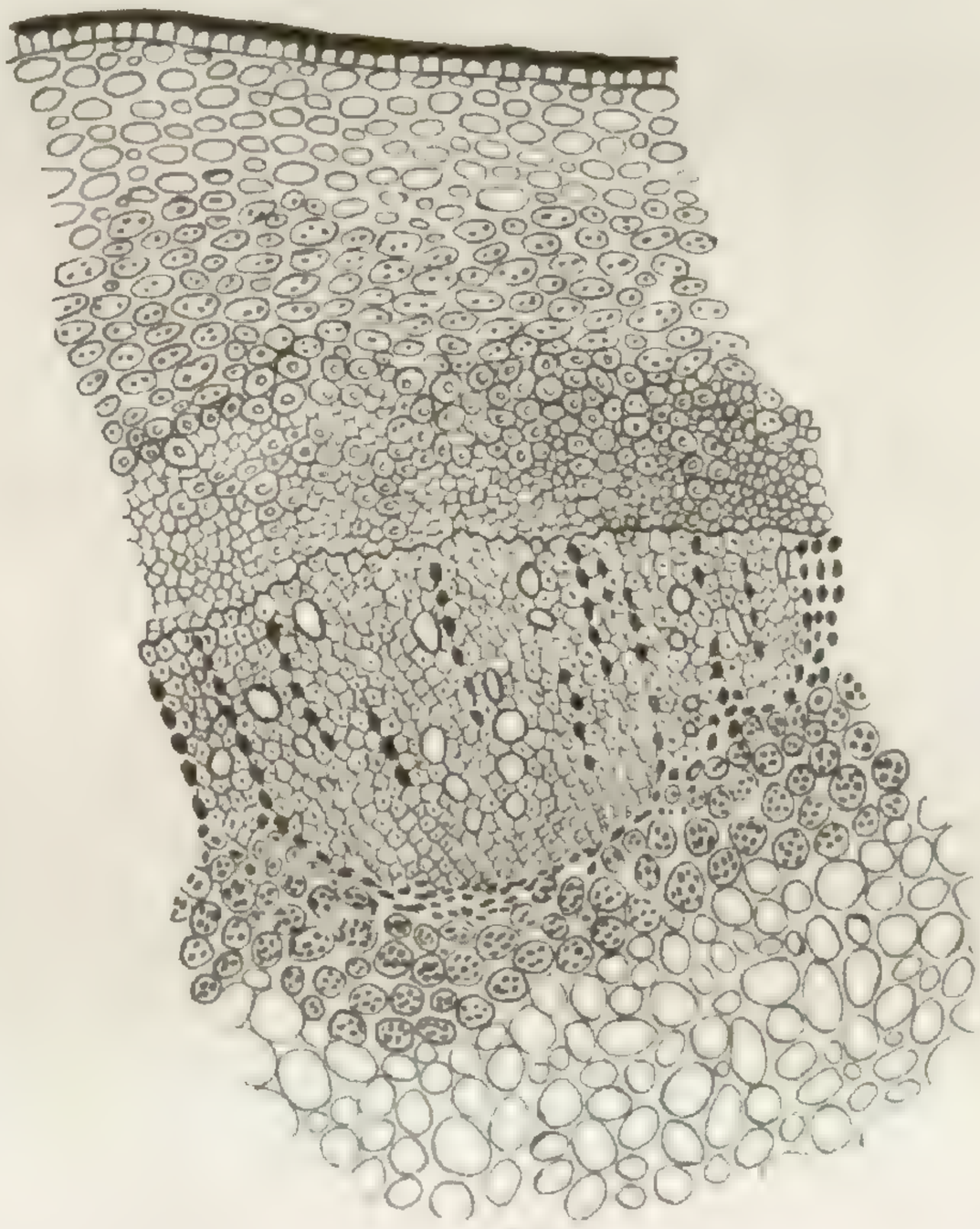

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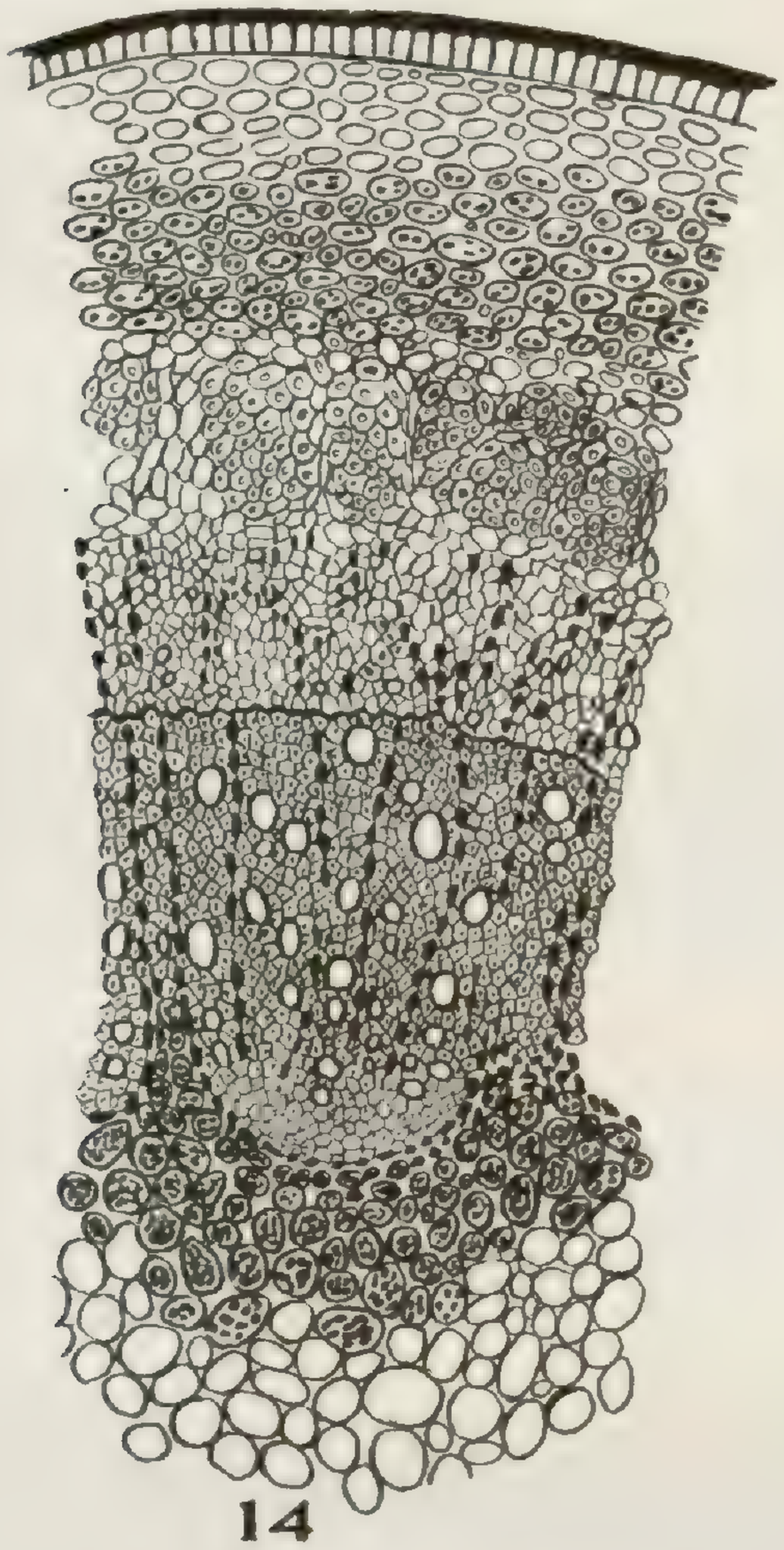
COOK on PEACH DISEASES



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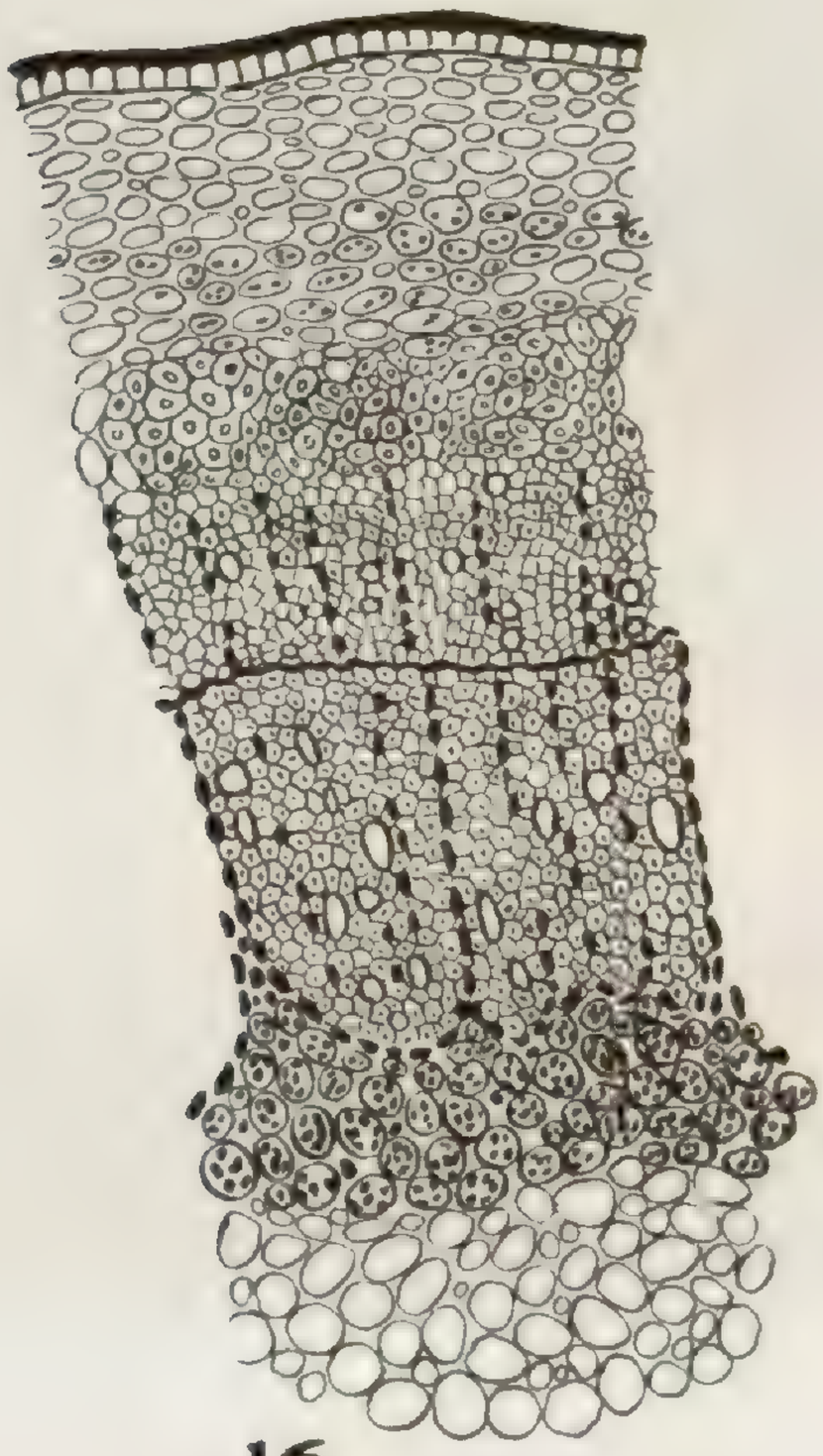

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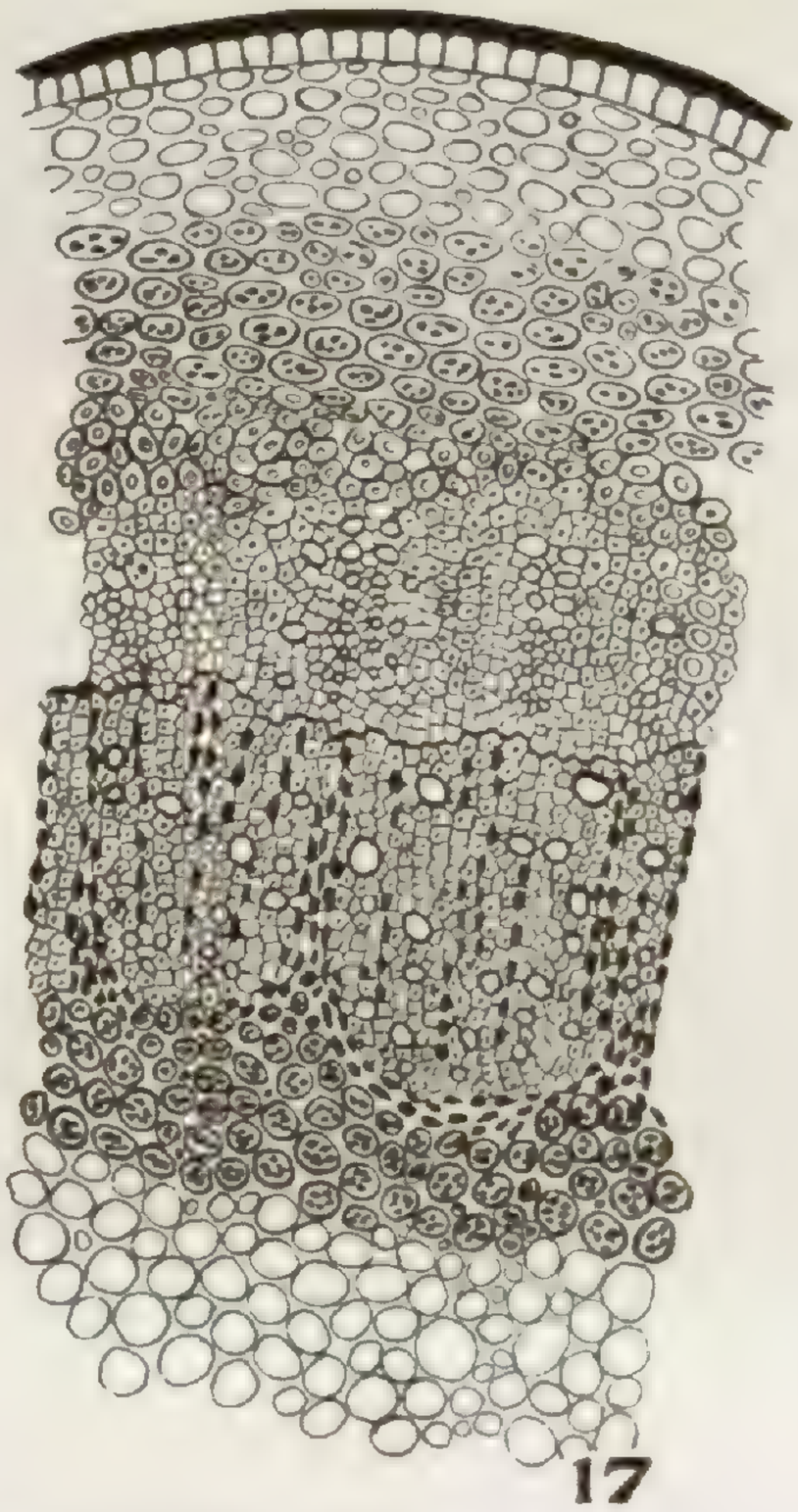

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COOK on PEACH DISEASES

The writer is indebted to Professor M. A. BLAKE and Mr. CHAS. H. CONNORS of the New Jersey Agricultural Experiment Station, for advice and assistance; and to Miss GERTRUDE E. MACPHERSON for cutting the sections and doing most of the work on the drawings.

RUTGERS COLLEGE
NEW BRUNSWICK, N.J.

EXPLANATION OF PLATES VI, VII

FIG. 1.—Normal leaf of Elberta peach taken early in morning.
FIG. 2.—Same taken in afternoon.
FIG. 3.—Normal leaf of Hiley peach taken in morning.
FIG. 4.—Same taken in afternoon.
FIG. 5.—Leaf of Hiley with yellows taken in morning.
FIG. 6.—Same taken in afternoon.
FIG. 7.—Leaf of Chinese cling with yellows, taken in morning.
FIG. 8.—Same taken in afternoon.
FIG. 9.—Leaf of Hiley with little peach taken in morning.
FIG. 10.—Same taken in afternoon.
FIG. 11.—Leaf of Elberta with little peach taken in morning.
FIG. 12.—Section of twig of normal Hiley taken in morning.
FIG. 13.—Same taken in afternoon.
FIG. 14.—Section of twig of Hiley with yellows taken in morning.
FIG. 15.—Same taken in afternoon.
FIG. 16.—Section of twig from Hiley with little peach taken in morning.
FIG. 17.—Same taken in afternoon.

EFFECT OF LOCATION OF SEED UPON GERMINATION

EDWARD N. MUNNS

The influence of parent trees upon the size and germination of Jeffrey pine seeds has been shown in a previous paper.[1] The marked results obtained from that work resulted in the present study, in which it has been sought to determine the value of seeds from different parts of the pine cone; and to decide what relation, if any, exists between the position of the seed and germination. The cones used were collected from *Pinus Jeffreyi* trees on the eastern slope of the Sierras in Lassen County, California, in September 1919. No attempt was made to choose the trees from which the cones were taken, except that the trees were young and growing thriftily, considering the site upon which they stood.

The cones were grouped according to size in three divisions, based on the gross characteristics of length, breadth, and weight. They were dried slowly in a room at air temperature, and as they opened the seeds were extracted. The cones were divided into three sections of approximately equal size, to be known as the upper, middle, and lower portions. The seeds were carefully collected and graded into three classes, large, medium, and small, using ocular means of determining the size and comparing one seed with another. Inasmuch as a number of individuals helped to determine the size of the seed grains themselves, the individual variation from this source was very largely eliminated. The seeds were cleaned, counted, and weighed, each lot kept separately, and sufficient seeds to carry out the test taken at random from each lot. To determine the germination, a number of each lot of seeds were sown in cans containing a uniform depth of soil and covered by an approximately equal depth of sand in each case. As previous work has shown that for Jeffrey pine a soil moisture content of about 15 per cent gives the best results, frequent weighings were made to keep the moisture content of the samples a constant at this figure. The result of this study is presented in tables I–VII.

[1] MUNNS, E. N., Effect of fertilization in seed of Jeffrey pine. Plant World 22:4. 1919.

It was found that there was an increase in the number of seeds with an increase in the size of the cones, medium cones having 27 per cent more seeds than the smaller ones, and the large cones

TABLE I

NUMBER AND WEIGHT OF SEED PER CONE

SIZE OF CONES	WEIGHT OF CONE (GM.)	NUMBER OF UNDEVELOPED SEEDS PER CONE	NUMBER OF DEVELOPED SEEDS PER CONE	WEIGHT OF DEVELOPED SEEDS IN CONE (GM.)	AVERAGE WEIGHT PER 100 SEEDS (GM.)						
					By portion of cone			By size of seed			Average for cone
					Upper	Middle	Lower	Large	Medium	Small	
Large	228.8	69.6	62.0	5.33	8.47	8.75	8.49	10.33	8.21	6.17	8.57
Medium	189.2	58.0	51.6	4.08	7.30	8.22	7.83	9.24	7.82	5.81	7.78
Small	145.7	37.3	38.9	2.66	6.42	7.18	7.68	7.83	6.49	6.19	7.09

TABLE II

NUMBER OF SEEDS PER CONE BY SIZE OF SEED

SEEDS	LARGE CONES				MEDIUM CONES				SMALL CONES			
	Upper	Middle	Lower	Total	Upper	Middle	Lower	Total	Upper	Middle	Lower	Total
Undeveloped	28.7	22.2	18.7	69.6	25.5	16.9	15.9	58.3	13.0	14.5	19.7	47.2
Large	4.9	10.2	3.6	18.7	6.2	6.9	1.8	14.9	13.6	1.5	2.0	17.1
Medium	13.2	16.1	6.4	35.7	7.3	14.9	5.6	27.8	6.5	2.9	4.6	14.0
Small	3.3	2.0	2.3	7.6	2.0	5.1	1.8	8.9	3.6	3.0	1.9	8.5
Total developed seeds	21.4	28.3	12.3	62.0	15.5	26.9	9.2	51.6	23.7	7.4	8.5	39.6
Total	.....	.....	.....	131.6	.....	.....	.....	109.9	.....	.....	.....	86.8

TABLE III

PERCENTAGE OF SEEDS IN CONE BY LOCATION IN CONE

SEEDS	LARGE CONES				MEDIUM CONES				SMALL CONES			
	Upper	Middle	Lower	Total	Upper	Middle	Lower	Total	Upper	Middle	Lower	Total
Undeveloped	41.2	31.9	26.9	100	43.7	29.0	27.3	100	27.5	30.7	41.8	100
Large	26.2	54.5	19.3	100	41.6	46.3	12.1	100	79.5	8.8	11.7	100
Medium	37.0	45.1	17.9	100	26.3	53.6	20.1	100	46.4	20.7	32.9	100
Small	43.4	26.3	30.3	100	22.5	57.3	20.2	100	42.3	35.3	22.4	100
Total developed seeds	34.5	45.6	19.9	100	30.0	52.2	17.8	100	59.8	18.7	21.5	100

51 per cent more than the small cones. Another interesting thing was that there were more undeveloped seeds than developed, except in the case of the small cones, where there was a slight decrease. In the large cones 47.1 per cent of the seeds were fully developed,

in the medium cones 47.0 per cent, and in the small cones 54.2 per cent. In each cone it was found that there were twice as many large as small seeds, and more medium seeds than there were large and small seeds together, except in the small cones where there was a slight decrease.

The quantity of large seeds amounted to about 30 per cent of the total in the large and medium sized cones, and 43 per cent in the

TABLE IV

PERCENTAGE OF SEEDS IN CONE BY DEVELOPMENT AND SIZE

SEEDS	LARGE CONES				MEDIUM CONES				SMALL CONES			
	Upper	Middle	Lower	Average	Upper	Middle	Lower	Average	Upper	Middle	Lower	Average
Developed	36.7	50.0	39.5	47.1	50.0	50.0	37.2	47.0	50.0	62.2	46.6	54.2
Undeveloped	63.3	50.0	60.5	52.9	50.0	50.0	62.8	53.0	50.0	37.8	53.4	45.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
				Total				Total				Total
Large	7.9	16.5	5.8	30.2	12.0	13.4	3.5	28.9	34.4	3.8	5.0	43.2
Medium	21.3	26.0	10.3	57.6	14.1	28.9	10.8	53.8	16.4	7.3	11.6	35.3
Small	5.3	3.2	3.7	12.2	3.9	9.9	3.5	17.3	9.1	7.6	4.8	21.5
Total	34.5	45.7	19.8	100.0	30.0	52.2	17.8	100.0	59.9	18.7	21.4	100.0

TABLE V

WEIGHT OF SEEDS IN GRAMS PER 100 SEEDS

SIZE OF SEED	LARGE CONES			MEDIUM CONES			SMALL CONES		
	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower
Large	10.20	10.14	11.07	8.35	9.80	10.15	7.57	8.33	9.87
Medium	8.47	8.15	7.84	7.11	8.07	8.08	5.32	7.60	7.15
Small	5.92	6.41	6.32	4.81	6.37	5.30	4.20	6.20	5.43

small cones; medium seeds made more than 50 per cent of the total in the large and medium cones, and 35 per cent in the small cones; while the small seeds formed 12 to 17 per cent in the large and medium cones, and 21 per cent in the small cones.

The weight of seeds ranged from 4.20 to 11.07 gm. per hundred seeds, the average being 8.28 for large cones, 7.56 for medium cones, and 6.85 for the smallest. Using the smallest seeds as unity, the heaviest seeds in the small cones exceeded this by 135 per cent, the smallest in the medium cones was 14.5 per cent

heavier, while the largest was 141.7 per cent. The lightest seeds in the large cones exceeded the smallest seeds by 41 per cent, and the largest seeds were 164 per cent heavier than the smallest seeds.

Table VI shows that it is the size of seed rather than position in the cones which is the determining factor, there being a decided decline in the germination per cent with the size, while apparently no relation holds between position and germination. It has been shown that the weight of the seed is directly influenced by the

TABLE VI

GERMINATION PER CENT BY SIZE OF SEED AND LOCATION

Material	Large cones	Medium cones	Small cones	Average
Large seed	66.9	58.1	55.2	60.1
Medium seed	51.9	52.4	35.4	46.6
Small seed	35.2	25.5	23.7	28.1
Upper cones	51.0	38.6	35.8	41.8
Middle cones	52.7	50.7	39.5	47.6
Lower cones	50.7	46.7	39.1	45.5
Average for cones	51.5	45.3	38.1	45.0

TABLE VII

GERMINATION PER CENT BY WEIGHT OF 100 SEEDS IN GRAMS

Weight per 100 seeds	Germination per cent	Weight	Germination per cent	Weight	Germination per cent
4.0	17.5	7.0	40.5	10.0	64.5
5.0	24.5	8.0	48.5	11.0	72.5
6.0	32.5	9.0	56.5	12.0	80.5

size of cone, and this is further reflected in the germination. Charting the weight and germination, it was found that a straight line relation existed, which is expressed in table VII. A curious relation was shown in the rapidity of germination. Seeds from the lower portion of the cones completed half their germination five days sooner than seeds from the middle third of the cone, which in turn were five days earlier than seeds from the upper part of the cone. Apparently this was independent of the size of the seeds and varied with the size of the cone, the seeds from the larger cone being the more rapid. Final germination apparently did not conform to any regularity, except that the seeds from larger cones

completed their germination first, followed by the medium sized cone, the small cone seeds being last, with two weeks difference between the large and small cones.

These results have an immediate application in forestation work. So far as is known, little attention is being paid to the parentage or the condition of the seed before sowing. As pointed out previously, only seeds from thrifty trees should be used, and in the present study it appears that if it is impossible to collect only the largest cones in the field, a screening process is necessary to remove the small seeds and secure only those of large size. Studies under way show a relationship between the size of seeds and the early growth and establishment of forest tree seedlings similar to that given here, and it is believed that the "dominance" classes in the forest in a measure are an index of the size of the seed from which the tree originated. To secure the best possible forest, it is believed that forest nursery practice should be confined only to the production of trees from the heaviest and therefore largest seed.

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CURRENT LITERATURE

BOOK REVIEWS

Diseases of economic plants

The appearance of a revised edition of STEVENS and HALL'S *Diseases of economic plants*[1] will be welcomed by every one interested in plant pathology. Since the publication of the first edition in 1910, so much progress has been made in the rapidly expanding field of plant pathology that a revised edition of this work will be appreciated, especially by the busy teacher and investigator.

The general plan of the book is similar to that of the first edition, although some changes have been made. The first fourteen pages are devoted to a brief summary of the history of plant pathology, the damage caused by plant diseases, and methods of prevention or cure. General diseases, such as damping off, root rot, and soil diseases, are discussed in a special section of the book. The diseases of special crops are grouped under the crop plants upon which they occur, and a chapter on tropical diseases has been added. This is followed by a chapter on fungicides and spraying apparatus, and another on soil disinfection. The bibliography contains 556 well chosen titles. Since the book is intended primarily as a text for college students, according to the author, many students, and certainly many teachers, will wish that the historical summary were more detailed. One might wish also that the damage caused by plant diseases had been discussed more fully. An account of the most serious epidemics probably would have been especially appreciated. The methods of disease prevention are grouped on the basis of more or less specific operations, such as seed treatment, the use of protective sprays and dusts, the selection of resistant varieties, and avoidance of practices which aid in the dissemination of the parasite. A brief account of quarantines possibly might have been desirable; and a more general grouping of control measures probably would seem preferable to some pathologists. The discussion of specific diseases is limited to essential facts. The economic importance signs, general etiology, and control measures are given for all important diseases. The presentation is as detailed as could be expected in a book of such wide scope, and the literature citations direct the student to sources from which further information may be obtained.

The book is an excellent compendium of practical facts regarding plant diseases, and should be especially valuable as a reference. It is concisely written, well illustrated, and contains an extensive bibliography. It is to be hoped that the book will find its way into the hands not only of students, teachers, and investigators, but also of farm bureau advisers and the more

[1] STEVENS, F. L., and HALL, J. G., Diseases of economic plants (revised edition by F. L. STEVENS). pp. 507. Macmillan Co. 1921.

intelligent growers. STEVENS has rendered a distinct service to phytopathology by summarizing in a compact, neatly bound volume such a vast body of knowledge in an increasingly important field of applied botany.—E. C. STAKMAN.

A textbook of botany

Under the title *General Botany*, DENSMORE[2] has added to the already numerous textbooks of elementary botany whose scope and content are suitable for use in the junior college or normal school. The headings of the first two and of the last chapter in the book, The relations of plants to the environment, The form and adjustments of the plant body to the environment, and Plant associations, show that ecology has been given due emphasis. The intervening chapters are devoted to plant anatomy, physiology, and morphology in a way that seems to fit the title of the book. There is even an attempt at the beginnings of classification, with the consideration of representative species and families from the spring flora. In a word, the material is sufficiently comprehensive that in the hands of a good teacher it will furnish the basis of a good general introductory course in the subject.

There is evidence in the volume that it comes as a result of a wide experience in the laboratory and in the field. The illustrations are numerous, many are original, and several, such as those of diagrammatic life histories, are of more than usual merit. The addition of a glossary would have supplemented the usefulness of the volume.—GEO. D. FULLER

West African forests

A volume entitled "West African forests and forestry," by UNWIN,[3] late conservator of forests, Nigeria, is principally concerned with the economics of the forests of the west coast of Africa from the Senegal to the Congo River. It includes the regulations restricting the cutting of timber, the efforts at reforestation, notes on the most valuable timber trees, and summaries of forest exports. There are also chapters on The oil bean seeds and nuts of the forest; The oil palm and palm kernel industry; The forest in relation to agriculture; and A bibliography of West African forests. Considerable space is also devoted to the native names for the various trees.

The abundance and excellence of the photographs, together with the notes on the general forest conditions, furnish the ecologist and geographer with considerable data regarding the forest formation in a relatively unknown region. The index seems to be adequate and able to add to the usefulness of the volume, but the bibliography leaves much to be desired in the way of accuracy and completeness of citations.—GEO. D. FULLER.

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[2] DENSMORE, H. D., General botany. 12mo. pp. xii+459. *figs. 289*. Ginn and Co., Boston. 1920. $2.96.

[3] UNWIN, A. H., West African forests and forestry. 8mo. pp. 527. *figs. 110*. London: T. Fisher Unwin Ltd. 1920.

NOTES FOR STUDENTS

Flora of southern Illinois.—In analyzing the elements entering into the flora of the southern portion of the state of Illinois, PALMER[4] distinguishes as the most notable feature the presence of typically southern species which here reach their most northern extension. This southern element he regards not as a recent invasion, but as the remnants of a more numerous aggregation that existed here in the remote past. These species, therefore, are not extending but rather restricting their range. Two floristic formations are distinguished and named the Cairo and Mounds formations from the towns about which they center. The former dominates the rich soils of the Mississippi and the Ohio River flood plains formerly covered with rich forests. Among the common tree species are *Taxodium distichum, Nyssa aquatica, Gleditsia aquatica, Fraxinus profunda, Liquidambar styraciflua, Quercus lyrata, Betula nigra, Carya laciniosa,* and many others. Among the herbaceous plants may be mentioned *Hottonia inflata, Triadentum petiolatum, Dianthera ovata, Spilanthes americana,* and *Mikania scandens.* The Mounds formation reaches its best development upon some low hills with gentle slopes of Cretaceous age. Its typical trees are less distinctively southern, and include such species as *Carya glabra, Quercus Muhlenbergii, Q. velutina, Q. Schneckii, Liriodendron tulipifera, Cercis canadensis,* and *Acer saccharum.* Upon the lower elevations the trees are large and tall, while upon the poorer soil and greater elevations of the Ozark hills not only is there a decrease in size, but there is a greater predominance of oaks and hickories, such as *Quercus velutina, Q. alba, Q. stellata, Carya glabra, C. ovalis,* and *C. alba.*

The report concludes with a list of woody plants collected. This includes not less than twelve species and varieties of *Carya* and fifteen species and eight hybrids of *Quercus.*—GEO. D. FULLER.

Seasonal changes in carbohydrates—MITRA[5] has recently published a paper on seasonal changes of carbohydrate materials in apple seedlings. Analysis has been made on one- and two-year old stems and roots and on fruit spurs, for the determination of the amount of starch, sucrose, maltose, glucose, and total sugars at intervals of fifteen days during the year. Some determinations of acidity in autumn, winter, and spring have also been made. Starch reaches its maximum amount in one- and two-year old apple stems in October and November, with a secondary increase in June. The same is true of roots. Total carbohydrates show a similar curve, reaching 44 per cent in winter. Total and reducing sugars in one- and two-year old stems and in roots increase in January and March. The author finds an increase in acidity in November.

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[4] PALMER, E. J., Botanical reconnoissance of southern Illinois. Jour. Arnold Arboretum **2**:129–153. 1921.

[5] MITRA, S. K., Seasonal changes and translocation of carbohydrate materials in fruit spurs and two-year old seedlings of apple. Ohio Jour. Sci. **21**:89–103. 1921.

while the tissue is practically neutral in February and March. He states also that there is a general correlation between acidity of tissues and the relative activity of diastase and maltase as determined from amount of glucose and maltose in tissues. Maltose is most abundant when acidity is high and near the optimum for diastase. Glucose is found to increase in quantity in the late winter at a time when tissues are practically neutral, acidity being near the optimum for maltase activity. An average of eight determinations of maltose made in November, when acidity is highest, is 1.99 per cent, and an average of eight similar determinations made in March, when tissues are practically neutral, shows 1.86 per cent maltose. This difference seems too insignificant to conclude that maltose is present in larger quantities at a time when acidity is highest, especially when maltose determinations vary from 0.46 to 3 or 4 per cent. The only conclusion concerning this, in the reviewer's judgment, is that maltose is always present and in very variable amounts.—JOHN M. ARTHUR.

Ecology of the Gangetic plain.—In a paper of more than usual interest, DUDGEON[6] has included the results of his studies of a region whose ecology has been almost unknown. This part of India, lying immediately about Allahabad, has a distinctly periodic climate, with about 90 cm. of rainfall, and three distinct seasons. The rainy season, from June to the end of September, has high precipitation, high humidity, high temperature, and low insolation; the cold season, from October to the end of February, has high humidity, high insolation, but low rainfall and low temperature (mean 35° F. to 55° F.); the third or hot season, has low rainfall and humidity, but high insolation and temperature (mean 80° F.).

The existing vegetation is shown to be influenced quite as much by the biotic factors of a human population of 530 persons and 470 domestic grazing animals per square mile as by the nature of the climate. Most of the area is covered with dry meadow and thorn scrub, but it seems certain that these associations, now balanced against intense human influence, are really the retrogressive remains of a much richer climatic vegetation. The author seems to have thoroughly established his final conclusion, that "if the retrogressive influence of the biotic (human) factors were removed, the vegetation would pass through the progressively higher forest stages of (1) fully developed thorn scrub, (2) pioneer monsoon deciduous forest, and (3) climatic climax monsoon deciduous forest, a forest of considerable density and luxuriance." This forest, as shown by adjacent regions, would show *Terminalia tomentosa* and *Tectona grandis* as dominant, and would also contain *Sterculia* spp., *Bombax malabaricum*, *Anogeissus latifolia*, *Buchanania latifolia*, *Eugenia jambolana*, and probably *Acacia catchu* and *Shorea robusta*.—GEO. D. FULLER.

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[6] DUDGEON, WINFIELD, A contribution to the ecology of the upper Gangetic plain. Jour. Ind. Bot. 1:1–29. *figs.* 9. 1920.

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DECAY OF BRAZIL NUTS

EDWIN ROLLIN SPENCER

(WITH PLATES VIII–XII AND THREE FIGURES)

Introduction

Brazil nuts, Para nuts, Cream nuts, etc., are the seeds of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl. The nuts are harvested in the months of January, February, and March, when the heavy pericarps containing the seeds fall to the ground. They are collected and transported from the forests to the seaports at a time of year when heat and moisture favor fungous growth, and often a cargo reaches New York 30 per cent spoiled. The United States Bureau of Chemistry holds that nuts are adulterated food if more than 15 per cent are spoiled, and requires that such nuts be shelled before being placed on the market. In spite of these measures, however, Brazil nuts reach the consumer containing from 10 to 25 per cent of spoiled nuts. There were 43,076,348 pounds of Brazil and Cream nuts shipped into the United States in 1919 (**7**). It is probable that half of this amount, or 21,538,174 pounds, were retailed in the shell. It is conservative to estimate the loss through spoiled nuts at 10 per cent of this amount, or 2,153,817 pounds, an approximate money loss, at 40 cents per pound, of $861,526.80, which falls directly upon the consumer. Brazil nuts do not become rancid very readily, and for this reason they are not placed in cold storage during warm weather as are most other nuts. They are heaped in piles in supposedly dry and often very

hot rooms where, when moisture is present, fungous growth is favored.

There is a wide difference in shell porosity of Brazil nuts, and a positive correlation between fungous infection and shell porosity has been demonstrated. Two two-pound samples of Brazil nuts purchased at two different grocery stores were tested for porosity of shell as follows. The nuts were taken one at a time and dipped first in 95 per cent alcohol to prevent the collection of surface bubbles, and then plunged two or more inches beneath the surface

TABLE I

Percentage spoiled	Very porous		Slightly porous		Least porous		Spoiled in sample
	Cracked	Spoiled	Cracked	Spoiled	Cracked	Spoiled	
	Sample I						
100	4	4					
34			26	9			
12					49	6	
25, in sample							19
	Sample II						
50	2	1					
38			21	8			
7					70	5	
15, in sample							14

of hot water contained in a tall beaker. The heat-expanded air arose in bubbles from the pores of the shell. Table I shows the results obtained. The conclusion to be drawn from these data is that the most porous nuts are not necessarily spoiled, but readily become infected when conditions favor infection, while the least porous nuts are much less subject to infection. It is quite possible that so long as the water content of the nut is sufficient to support fungous growth, nuts with very porous shells may be entered and spoiled if storage temperature is favorable. Such infections probably account for the high percentage of spoiled Brazil nuts bought of retailers whose wholesale patrons have scrupulously complied with the ruling of the Bureau of Chemistry when the nuts were purchased at port.

Although the use of nuts as foods and confections has recently become extended and general, there is but little concerning nut diseases in the literature, and studies of the diseases affecting the nuts only for the most part have been superficial. MANGIN (**17**) described a "black rot" of chestnuts caused by *Harziella castanae* Bain., and found it to cause a 26 per cent loss of nuts gathered late in the season. VON IVANOFF (**31**), in studying *Trichothecium roseum* Link, found it in pure state on the kernels of *Corylus avellana* and *Pinus cembra*, and this, with RAND'S (**23**) work on *Coniothyrium caryogenium* Rand, is the only serious investigation of nut parasites that has been made. MARTZ (**18**) reports a species of *Cephalothecium* on pecans in Florida. KUHL (**13**) isolated *Aspergillus flavus* Mont. from Brazil nuts, but his description of both disease and fungus is meager.

A few parasites of nut plants cause diseases of the nuts themselves. The most serious disease of this kind is that produced by *Pseudomonas juglandis* Pierce, which, according to SMITH (**27**), attacks the nuts as well as other growing parts, and "a nut in such cases is deformed in shape . . . . and the kernel . . . . is only poorly developed." PIERCE (**21**) says that in young nuts the kernel is destroyed. Chestnuts are affected by *Endothia parasitica* (Murr.) A. and A. RUMBOLD (**24**) says that the hyphae of this parasite spread throughout the kernel. The kernel spot of pecan produced by *Coniothyrium caryogenium* Rand has incidently been studied by TURNER (**30**), and by RAND (**23**). In addition to these studies, there have been some reports on storage results (**6, 29**), and MCMURRAN (**16**) mentions what he considers a non-parasitic disease, the "black-pit" of pecan.

The aim of the present investigation was to isolate and identify as many as possible of the more important fungi and bacteria causing deterioration of Brazil nuts. Seven distinct organisms have been isolated, studied, and their etiological relation to the nut deterioration demonstrated. The remainder of the paper comprises the methods of study and descriptions of the organisms isolated.

Methods

The nuts studied were obtained from two wholesale firms in Chicago and from retail grocery stores in Champaign and Urbana,

Illinois, and were purchased at different times during the year 1919–20. Each nut was superficially examined and the shell carefully removed by cracking it with a hammer. The diseased nuts were dropped, shell and all, into suitable sterilized glass dishes, one nut in each dish. A number was assigned to each, but only those diseases which were most prevalent and which presented the most conspicuous diagnostic features were selected for study.

A preliminary examination was made of thin razor sections of diseased tissue mounted in water or xylol, in order to discover whether fungi or bacteria were present, and if so, to ascertain their general relation to the host tissues. If this examination showed any single species of organism to be predominant, isolations were made either by direct transfer to cornmeal agar plates, or by dilution plating as the case required. These isolations were from both exterior and interior portions of the nut, and when from the interior were carried out in the following manner. The nut meat was cut into with a flamed scalpel and carefully broken apart. A central portion of about 4 sq. mm. area was carefully removed with a flamed scalpel from one of the newly exposed surfaces, and discarded. In the center of the cavity thus made small pieces of diseased tissue were loosened with the point of the scalpel, and immediately carried in a sterilized loop to the surface of cornmeal agar plates.

Following isolation, the next step was to determine whether the fungi isolated were responsible for the various diseased conditions. Two methods were used; first, pieces of mycelium or a few spores were placed on sterile kernels contained in sterilized one-inch test tubes; and second, pieces of mycelium or a few spores were placed upon strips of sterile nut meat, 50 $\mu \times 5 \times 10$ mm., which were contained in tubes of sterile water, one strip on the side of the tube just above the surface of the water and the other in the water. By the first of these methods the rotting power of the parasite was made evident within a few weeks by the softening of the whole mass. With the second method results were obtained more quickly by more or less complete dissolution of the very thin sections employed. The following media were used in the case of every organism.

CORNMEAL AGAR.—This was prepared as recommended by SHEAR (**26**), except that the medium ready for filtration was poured into precipitation cones and autoclaved. After solidifying, the precipitated dirt at the apex of the inverted cone was removed and the clean agar melted and tubed.

BRAZIL NUT AGAR.—Fifty grams of Brazil nut kernels which were free of, or easily freed from, their inner seed coats were ground in a nut grinder (a Russwin no. 1 Food Cutter was used), and steeped for one hour at from 58° to 60° C. in 1000 cc. of distilled water, counterpoised, and filtered. Fifteen grams of powdered or crude agar was added and the mixture boiled for ten minutes, counterpoised, poured into precipitation cones, and autoclaved at 15 pounds for fifteen minutes. After solidification the agar was removed from the glass cone and placed on a clean sheet of paper. After removing the sediment the dirt-free agar was returned to the precipitation cone and again autoclaved. The resolidified agar cone was in two distinct layers, and the translucent layer was the one used.

NUT PLUGS.—It was found possible, by flaming a scalpel after each stroke, to cut out nut plugs of considerable size which were free from contamination. The kernels from which such plugs were to be cut were placed in a 2 to 1000 solution of mercuric chlorid where they remained for thirty minutes. They were taken from the solution one at a time, held by one end between thumb and finger, and shaped by cutting away a thin layer with a sharp scalpel, flamed after each stroke. When the plug was finished it was cut off after placing it within the mouth of the reclining one-inch test tube. Nut plugs made in this way remained uncontaminated for several months.

NUT STRIPS.—Strips of nut meat, 50 $\mu \times 5 \times 10$ mm., were cut on a microtome and preserved in absolute alcohol. When used they were taken from the alcohol with sterilized forceps and placed in sterile water in a Petri dish. From this they were removed with a sterilized loop and placed in test tubes containing sterile water, as already described.

AUTOCLAVED RICE.—This medium was made by placing two or three grams of rice and twice the volume of water in test tubes and autoclaving.

MICROTOME SECTIONS.—These were made in order to show the morbid histology in comparison with the normal histology. Because of the oil content of the nut, ether was found to be the best killing and fixing agent. The ether was replaced after three days with chloroform, and the ordinary schedule for imbedding with this reagent followed (**2**). After sectioning it was found that the oil content had not been sufficiently lessened, and that without its removal a distinct view of the structures could not be secured. To obviate the difficulty the sections were fixed to the slide, treated with xylol, xylol and absolute alochol, absolute alcohol, and then flooded four or five minutes with ether. The slide was held horizontally between thumb and finger, and the dissolved fats collected on the under side of the slide, from which they were wiped off before placing the slide in 95 per cent alcohol. The slide was kept in each of the 95, 85, and 70 per cent alcohols for about five minutes, and then flooded with Pianese IIIb for 15 minutes (**32**), washed with distilled water, 70, 85, and acidulated 95 per cent alcohol, 95, 100 per cent alcohol, 100 per cent alcohol and xylol, xylol, and mounted in balsam. The stain shows the host tissue in red and the fungus in green.

Two of the fungi produced pycnidia which were sectioned for study. These were taken from cultures on cornmeal agar, killed and fixed with chromacetic fixing fluid, and stained with Bismark brown, following the usual schedule for this stain. The pycnidia were removed from the culture on a strip of agar, usually about $1 \times 2 \times 4$ mm. in size, which remained intact throughout the process and served well in the orientation of the specimens in the imbedding dish.

FREEHAND SECTIONS.—It was occasionally found necessary to stain razor sections made for the preliminary study of the diseased tissue. The sections were cut as thin as possible and placed in a watch glass contained in a Petri dish. The watch glass was then filled with ether and the Petri dish closed. When the ether had evaporated, 95 per cent alcohol was poured over the sections and allowed to stand for ten or fifteen minutes. This was followed with 85, 70, and 50 per cent alcohols for five minutes each. The sections were then transferred to slides prepared with albumen

fixative, flooded with water, and allowed to stand over night. They were stained with Pianese IIIb, or with jod grün-erythrosin in the following manner. They were placed in 95 per cent alcohol for five minutes, flooded with jod grün (1 per cent solution in 95 per cent alcohol) for thirty minutes, washed with 95 per cent alcohol, then absolute alcohol, flooded with 1 per cent solution of erythrosin in clove oil for forty-five minutes, washed with absolute alcohol, cleared with carbol-turpentine clearer, and finally washed in xylol and mounted in balsam. This proved to be the most satisfactory of any method tried for staining mycelium in the tissue.

PROTEOLYTIC ENZYMES.—The Brazil nut agar serves well to show the presence or absence of certain extracellular proteolytic enzymes. The proteid precipitates to which the opacity of the agar is due are digested by the enzymes, and a transparent halo, which enlarges as the thallus or colony enlarges, surrounds the growth. All the organisms studied were tested for the presence of these enzymes. The enzymes were precipitated from cornmeal broth in which an *Actinomyces* or a *Bacillus*, the two most active enzyme producers, was grown. The broth was poured into Piorkowski culture flasks to a depth of 0.25 inch, about 200 cc. being required for each flask, and inoculated. After ten days the culture was filtered through paper and enough 95 per cent alcohol added to the filtrate to make 80 per cent alcohol. Three days later a fluffy white precipitate had collected at the bottom of the precipitation cones, and the excess alcohol was siphoned off (**12**). From 25 to 50 cc. of absolute alcohol was added to the precipitate and immediately filtered. Before the precipitate had become dry it was again washed with 50 cc. of absolute alcohol, and while still moist was removed to a desiccator containing sulphuric acid, and allowed to remain there for two days. The hard, gray material was then scraped from the paper to be redissolved in sterile water when used.

MORPHOLOGY OF BRAZIL NUT.—The kernel of the Brazil nut, as it is ordinarily removed on cracking the shell, is covered with a thin, dry coat which may be quite loose or may adhere very tenaciously. The embryo within, principally radicle, is completely

enveloped with a layer of endosperm 40 to 50 μ in thickness, and as reported by YOUNG (**35**) is "plainly differentiated into cortical and medullary tissues separated by a layer of procambium along which rudimentary vascular bundles are arranged at intervals." There is a single, somewhat irregular layer of epidermal cells just beneath the endosperm, and within 5 mm. of the distal end are the two very minute, unequal cotyledons which "measure about 750 by 175 μ" (**1, 25**). The cortical and medullary cells are similar in shape and size, and are largely filled with oil and proteid bodies. The endosperm cells, arranged with the long diameter at right angles to a median plane, are especially rich in proteids (**28**). The procambium cells with the long diameter at right angles to that of the endosperm cells contain few or no proteid grains (fig. 1).

The outer seed coat or shell is made up of two layers; the outer with its crinkled surface is light brown and softer in texture than the inner layer, which is dark brown and has a glazed inner surface. In the angles of the shell this layer seems to be of two layers which divide, leaving spaces filled with still another tissue that is lighter in color and softer in texture than the outer of the shell layers. In the micropylar angles of the seed is a narrow cavity. Such cavities are termed by BERG (**1**) the "loculi spurii in testa," and extend the entire length of the shell. This open channel probably serves as the usual entrance of the parasites of the nut (fig. 1).

The tissues of the seed, taken in order, beginning with the shell are: (1) outer seed coat in two distinct layers, with a softer tissue filling the triangularly prismatic corners; (2) the thin inner seed coat which may or may not adhere to the kernel; (3) the endosperm layer, two cells thick; (4) epidermis, a single-celled layer; (5) cortex, of large storage cells; (6) procambium layer, generally three cells thick; (7) the medullary tissue, of large storage cells.

Diseases of the Brazil nut

1. BLACK CRUST

GENERAL DESCRIPTION.—Fully 5 per cent of all diseased Brazil nuts are affected with black crust, but there is no external indication of their condition, since the shells are normal in color and the

weight is the same as that of sound nuts. When the shell is removed the kernel presents a dull black appearance which, if the whole nut is affected, reminds one of a large sclerotium. A cross-section of the diseased kernel shows the blackened portion to consist of a thin layer, 100–250 μ in thickness, apparently having no connection with the tissues beneath, which, aside from their light brown color and their pronounced nut odor, appear to be normal

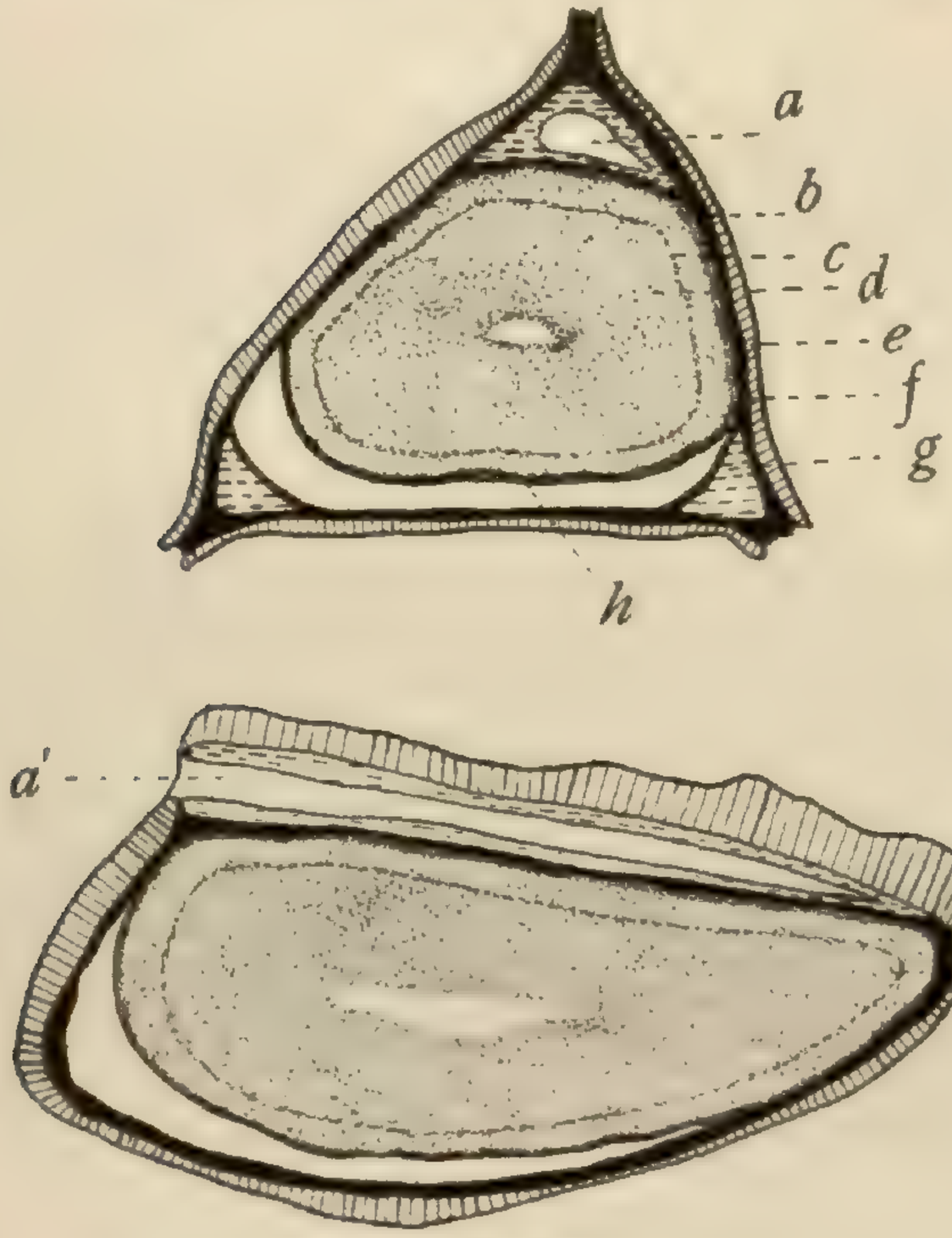

FIG. 1

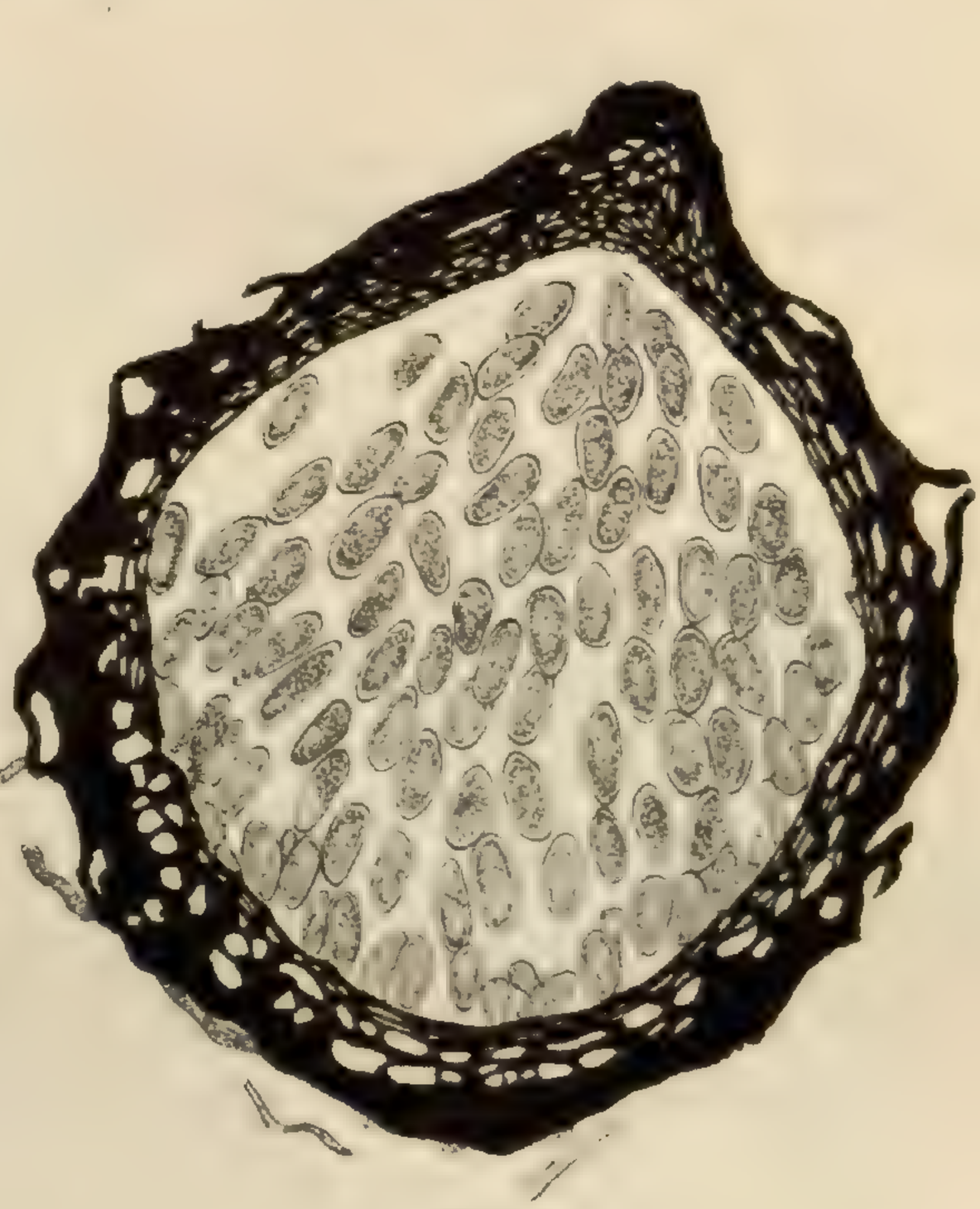

FIG. 2

FIGS. 1, 2.—Fig. 1, Diagrammatic drawings of cross and longitudinal sections of Brazil nut: *a* and a^1, locule in testa; *b*, endosperm layer; *c*, procambium layer; *d*, medullary tissue; *e*, outer layer of outer seed coat; *f*, inner layer of outer seed coat; *g*, soft tissue filling corners of shell; *h*, inner seed coat; fig. 2, pycnidium with immature spores, *Pellioniella macrospora* n. sp.; ×500.

(figs. 34–36). The diseased nut meats are frequently found covered with various fungi, chiefly *Penicillium* or *Aspergillus*, with black crust under the mold. A study of microtome sections shows that the mycelium is in the endosperm layer, the affected cells of which are hypertrophied (figs. 34, 35). The cortical cells of the radicle immediately beneath are not parasitized, but their contents are markedly changed. The proteid grains are almost or quite lacking in the epidermal and outer layers of the normal cortex, while in diseased nuts there is a superabundance of small proteid grains in

this region (figs. 34, 35). As it is possible to find nuts seemingly free from any other organism, the black crust fungus is easily isolated. Small pieces of tissue taken aseptically from immediately below the crust on direct plating gave pure cultures.

MORPHOLOGY.—The mycelium on cornmeal agar is of two kinds, that made up of cells which are longer than wide, and that with cells either nearly globular or wider than they are long (figs. 18, 19). The long-celled type predominates, both in the aerial and the submerged mycelium, except near pycnidia, where the shorter cells are most in evidence. The long cells are 14–32 μ in length by 3.5–14 μ in width, the short ones measuring 10–18 μ in diameter. Both types are thick-walled and black when mature, and both have granular contents (figs. 19, 20). In autoclaved rice and in the black crust of diseased nuts the hyphal cell is transformed until the hyphae suggest chains of conidia (figs. 14, 20). These cells are black, 10–15×5–8 μ in size, and contain one or two guttulae. They readily break away from the hyphae and function as spores.

Pycnidia are produced sparingly, and only along the border of a thallus where it comes in contact with another thallus, either of the same or of some other species. No pycnidia were found on diseased nuts or on any of the cultures except those on cornmeal agar plates. They are black, smooth, globose-conical, beaked, and 150–350 μ in diameter. The beak is 100–250 μ in length (figs. 2, 21).

The spores are borne at the base of the pycnidial cavity on short, hyaline, often septate conidiophores which are interspersed with narrow strap-shaped, hyaline, continuous paraphyses that are from one to six times as long as the conidiophores, the conidiophores being 5–14 μ in length by 3–5 μ in width (fig. 13). The spores are at first hyaline, unicellular, 26–36×14–20 μ in size and irregular in shape, but with maturity they become sooty black, striated, uniseptate, regular in shape and uniform in size, being 28×14 μ (fig. 15).

CULTURE CHARACTERS.—On cornmeal agar plates the fungus grows at the rate of 0.5–0.7 mm. per day at room temperature. The thallus is at first milk white, and the margin of it remains uncolored so long as it is increasing in size. After five or six days

the central portion becomes green, and a few days later turns sooty black. The thallus then is made up of three concentric rings, the outer white, the next green, and the innermost black (fig. 11). As the thallus ages it shows marked zonation, and becomes entirely black when growth ceases. Aerial mycelium is produced on all parts of the thallus, but is most luxuriant in the central area. On Brazil nut agar the growth is very much slower than on cornmeal agar, usually 0.3 mm. per day at room temperature, and the entire thallus remains hyaline. On nut plugs the growth was very weak, but a crust similar to that of naturally diseased nuts was formed after three months. On autoclaved rice the growth was vigorous, and several characteristic color reactions were noted (fig. 8). Eight days after inoculation: aerial mycelium snow white with line of Antique Green[1] below; rice grains in contact with glass, white bordered with Cerulian Blue; all interstices with greenish tints. After fifteen days: aerial mycelium white with lower border line Prussian Blue, almost black; contacts of grains with glass white bordered with Prussian Blue; interstices purple tinged.

The hyaline immature spores as well as the black mature ones germinated readily. The immature spores occasionally germinated in ten minutes after planting, while more than an hour is required for the germination of the mature spores, but the germ tubes of the mature ones soon outstrip those of the immature (figs. 16, 17). There is no change in either spore, except a slight swelling in germination, the immature spore remaining unicellular. This phenomenon of the germination of immature as well as mature spores has been pictured by HIGGINS (11) for a related species.

TAXONOMY.—The morphological characters of the fungus are those of *Pellioniella* Sacc., but according to SACCARDO (25) there is but one species in the genus, *P. deformans* Penz. and Sacc., whose spore measurements are a little more than half those of the Brazil nut parasite. The fungus, therefore, is given the name *Pellioniella macrospora*.

[1] The nomenclature used in describing colors throughout these investigations is that given in ROBERT RIDGEWAY'S Color standards and color nomenclature, published by the author, Washington, D.C. 1912.

Pellioniella macrospora, n. sp.—Pycnidia sparse, smooth, carbonaceous, globose-conical, beaked, 150–350 μ in diameter, beak 100–250 μ in length. Conidiophores at base of pycnidial cavity, hyaline, often septate, 5–15×3–5 μ. Paraphyses hyaline, strap-shaped, continuous, 5–50 μ. Immature conidia hyaline, unicellular, irregular, 26–36×14–20 μ; mature conidia sooty black, striated, uniseptate, regular, 28×14 μ.

HABITAT.—Parasitic on endosperm of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl.

2. WHITE MOLD

GENERAL DESCRIPTION.—White mold is not so common as black crust, and is probably responsible for less than 1 per cent of the Brazil nut decay, but it is a real factor in this loss. The diseased nut is normal in external appearance, but is below normal in weight. When cracked, the white, fluffy mycelium is seen to cover the entire kernel, but soon after exposure the hyphae collapse and the yellowed endosperm becomes visible through the mycelial mass. A pronounced musty odor arises from the newly shelled nut, but the taste of the diseased meat has nothing to distinguish it. A cross-section of the nut kernel shows three typical features of the disease: (1) the white moldy covering; (2) the endosperm layer, sulphur-yellow in color and more than twice as thick as in the normal nut; and (3) irregular cracks and cavities in the radicle, all filled with white mycelium and spores. The mycelium penetrates to the center of the radicle. The hyphae in the tissue are very tenuous, less than 2 μ in diameter, and are usually so closely associated with the cell walls of the host tissue as to make a study of them *in situ* very difficult, but the cell walls of the diseased nut are penetrated by them. The fungus was isolated as described, but spore dilutions made by touching a sterile loop to the mycelial mass in the internal check of the kernel gave pure cultures also.

MORPHOLOGY.—Mycelium taken from the nut, from nut plugs, and from other media was uniform in character. The following description is of mycelium taken from cornmeal agar plates. The cells measure 20–70×3.5–10.5 μ, and are hyaline with granular contents and guttulae. Anastomosis of cells is of frequent occur-

rence, especially in the submerged hyphae, while in the aerial mycelium simple loops and coils are common (fig. 24). The hyphae are unconstricted at the septa, and unbranched cells are of quite uniform size throughout their length. Cells bearing branches are swollen at the points from which the branches arise.

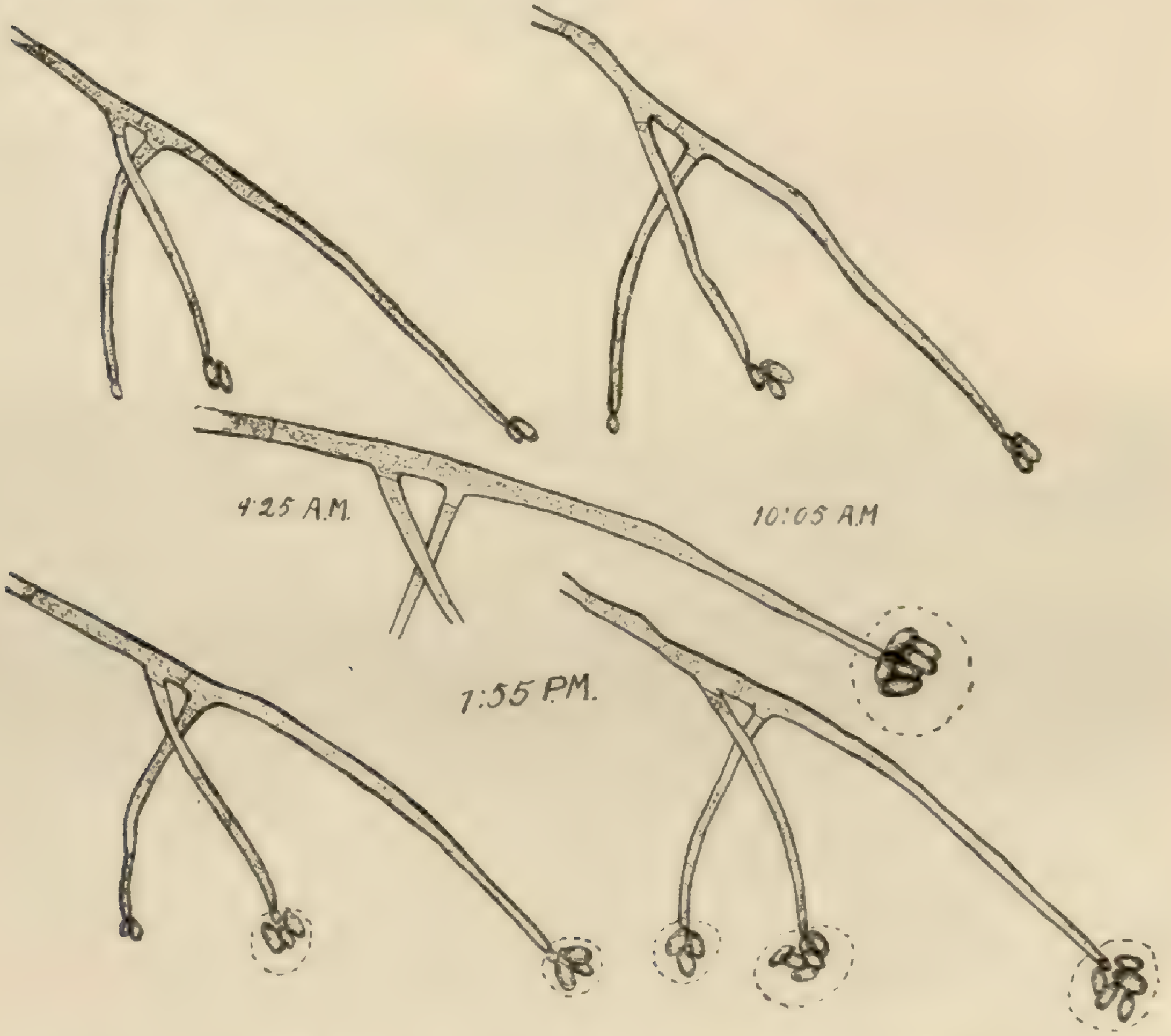

FIG. 3.—Development of spore mass of *Cephalosporium bertholletianum* n. sp.; dotted line shows size of water drop surrounding mass; ×100.

The conidiophores, 50–90 μ in length, are in most instances simple branches from either a principal filament or from its branches. They are spindle-like, with rounded tips from which the spores are cut off (fig. 3). The single-celled, oblong-elliptical, hyaline conidia are 8–12×3.5–54 μ in size, and contain two guttulae (fig. 23). They collect, as they are produced, in a spherical mass at the end of the conidiophore. A drop of water surrounds the spore mass after the third spore arrives (fig. 3).

CULTURE CHARACTERS.—On cornmeal agar the fungus grows rather rapidly, 0.5–1.0 cm. daily at room temperature. The thallus is very regular and is distinctly zonated after reaching a diameter of 4 or 5 cm. There is a dense growth of white aerial mycelium on the older portions of the thallus. On Brazil nut agar the characters are as previously stated, except that the growth is a little more rapid, and a halo 3 mm. wide, due to the digestion of the solid proteids, surrounds the thallus. On nut plugs the fungus grows luxuriantly and destroys the nut meat without giving off any appreciable odor. A large amount of fluffy mycelium is the characteristic feature of its growth on nut plugs as it is on the nut in the shell. On autoclaved rice the growth is vigorous and a pink tinge appears in the medium after two days. After ten days four colors are distinguished; where the rice is in contact with the glass in the older portions, Ochraceous Buff, in the younger, Venetian Pink; interstices between the grains are filled with mycelium through which a Jasper Pink color shows, in the older portions; in the younger portions it is Light Hortense Violet.

The nut strip above the water is soon covered with the dense mycelium, and is appreciably shrunken within five days. The strip in the water remains intact, but the water is soon filled with the mycelium which makes its way upward from the strip at the bottom. In hanging drop the spores germinate with a single germ tube, which in the most vigorous, at room temperature, may attain the length of the spore in two hours after planting. Spore production in hanging drop at room temperature proceeds at the rate of about one spore per hour per conidiophore. The conidiophore lengthens and increases in diameter as the conidia are cut off at the end. The process is very like spore production of *Trichothecium* as described by LINDAU (**14**).

TAXONOMY.—The fungus evidently belongs to *Cephalosporium*, but none of the species of this genus as reported by SACCARDO (**25**) has characters sufficiently like the Brazil nut parasite to permit it to be classified as one of them. *C. fructigenum* McAlp. (**15**) has spores of almost identical shape, size, and appearance, but it has knobbed conidiophores and oblong spore masses which are not present in this species, which therefore is described as new.

Cephalosporium bertholletianum, n. sp.—Conidiophores hyaline, simple or dichotomously branched, 50–90 μ long, 2- to 4-septate; spore mass globular; conidia hyaline, unicellular, oblong-elliptical, guttulate, 6–12 × 3.5–5 μ, ends obtuse.

HABITAT.—On radicle of seed of *Bertholletia nobilis* Miers and *B. excelsa* Humb. and Bonpl., causing decay.

3. DRY ROT

GENERAL DESCRIPTION.—The shell of the nut affected by dry rot is mottled, but of somewhat lighter shade in its darkest areas than the shells of normal nuts, and the weight is much below normal. The cracked shell appears to be filled with a kernel which adheres more closely to the shell than is usual, but which is so similar in color and general appearance to that of sound nut kernels that it might easily pass casual observation as such, although in reality it is merely a mass of mycelium. Small pieces of mycelium taken from this mass swell to approximately twice their size when placed in water. Under the microscope the hyphae were seen to be irregularly branched and septate. No conidiophores were seen, but what appeared to be unicellular elongated conidia of greatly varying length were occasionally found.

MORPHOLOGY.—The hyphae which make up both the aerial and the submerged mycelium are irregularly branched, and more or less constricted at the septa. The cells are 14–90 μ long by 3.5–11 μ wide, hyaline with granular contents and guttulae (fig. 44). Anastomosis frequently occurs, especially in older thalli, when spores falling on the medium germinate, producing a tube which unites with the cell of an older hypha, another germ tube, or another spore (figs. 29, 48).

The simple conidiophores are borne at any place along the hyphal strand, seldom more than two being produced by a single cell. Branched conidiophores are rare. Thalli resulting from direct planting of mycelium taken from the diseased nut produce but few conidiophores, and rarely more than single-celled conidia. Conidia from transferred cultures are from 1- to 8-celled, subcylindrical, slightly sickle-shaped, without pedicel, and conical at base (fig. 42).

No perithecia were found, but sclerotia were formed on autoclaved rice. These are dark gray and 500–1000 μ in diameter. Terminal chlamydospores are produced on 60-day old cultures. They are globular or oblong, with an average mean diameter of 14 μ, and with a scarcely perceptible yellow tinge (fig. 45).

CULTURE CHARACTERS.—Pure cultures are easily obtained by directly planting pieces of mycelium taken from the innermost portion of the mycelial mass. On cornmeal agar the rate of growth averages 0.5 cm. daily at room temperature. The thallus is arachnoid and regularly zonated, the zones averaging 0.5 cm. in width. Aerial mycelium covers the entire thallus but is most luxuriant in the central area, and there it is tufted. On Brazil nut agar the growth is more dense and a little more rapid than on cornmeal agar, its rate being from 0.7 to 1.0 μ at room temperature. An extra-cellular, proteolytic enzyme is secreted, causing a halo of 3–5 mm. in width in the medium surrounding the thallus. The aerial mycelium is more luxuriant on this agar than on the other.

The fungus grows vigorously on nut plugs, so that in a few days the plugs are enveloped with the snow-white mycelium, while a putrid odor is exhaled. After two or three months the plugs are completely reduced and only a mycelial mass remains. There is no color change on autoclaved rice until it shrinks away from the tube, when it is Maize Yellow. The fluffy, white, aerial mycelium surmounts the rice column and covers its sides as the growth proceeds downward. Apparently complete destruction of the rice is accomplished within two months. On carrot plugs the growth is not so rapid as on other media, but is marked by an abundance of white aerial mycelium. It is without color change. On the strip of nut meat above the water the growth is vigorous, and if the water surface remains near enough to the strip it is destroyed within eight or ten days. The strip in the water often appeared to be intact when it was not, the mycelium retaining the outline. It was probably destroyed as soon as the strip above.

In hanging drop the conidia begin germinating after three or four hours at room temperature, but many of them require twenty-four hours or more. Seldom more than two cells of a spore germinate, but frequently one cell produces two germ tubes (fig. 46). It often happens that spores are united by a short germ tube. Occa-

sionally four or five conidia are connected in this way (fig. 47), resulting, as is clearly shown by drop cultures, from germination succeeded by anastomosis (fig. 48). In all cultures the dense mycelium collects and retains water enough to germinate the 1- and 2-celled spores, and their germ tubes anastomose readily with the first cells, conidial or hyphal, with which they come in contact. It is often difficult to distinguish between conidiophores bearing conidia and conidia anastomosed to hyphal cells with a short germ tube (fig. 44).

TAXONOMY.—The fungus is a species of *Fusarium* which, according to WOLLENWEBER'S (34) scheme of classification, belongs to the section Eupionnotes: chlamydospores present; perithecia unknown; conidia subcylindrical, sickle-shaped; base without pedicel, conical; terminal chlamydospores.

4. ASPERGILLUS DECAY

GENERAL DESCRIPTION.—Brazil nuts attacked by *Aspergillus* may give no external indication of their internal condition except in the most advanced stages of the disease, when the weight of the nut is appreciably lowered. The kernel shrinks, often cracks open, and is always covered with a mass of dark brown spores. The odor of the diseased nut is strongly rancid with a putrid taint; the taste is at first sour, later very bitter. Occasionally nuts that are merely discolored have this same taste. KUHL (13) states that Brazil nuts affected with *Aspergillus flavus* Mont. are poisonous, and that the discoloration caused by this fungus is so slight that it does not prevent their being eaten. Both his observations and my own indicate that the disease, although present, may often escape notice, and that it is really far more prevalent than it appears to be under superficial examination. Nuts in advanced stages of the disease, however, occur less frequently than black crust. The mycelium of the fungus penetrates the tissues to the center of the nut, and when there is a central locule, appears as a white mold on the walls of the locule. When the diseased kernels crack open, a mass of spores fills the locular space.

MORPHOLOGY.—The mycelium consists of irregularly branched hyphae which are slightly constricted at the septa (fig. 31). The cells are 20–65 × 3.5–11 μ, with granular contents of a faint greenish

tint. Conidiophores varying from ten to several hundred microns in length arise at irregular intervals from the hyphae. The shortest of these have little or no filamentous part, but consist merely of the head and sterigmata (fig. 28). Sterigmata are also borne singly and in groups of from two to four on the hyphal cells (fig. 27). The heads of the conidiophores measure 10–20 μ in diameter, and the sterigmata, from two to many per head, are 10–12 × 5–7 μ. The globular, echinulated conidia are of different shades of yellow, and 5–10 μ in diameter, but the predominant size is 7 μ (fig. 29).

CULTURE CHARACTERS.—On cornmeal agar the rate of growth varies from 0.3 to 1.0 mm. daily at room temperature, and after forty-eight hours the central portion of the thallus shows the forming spore clusters in Light-Buff. The spore masses become darker with age until Lemon-Chrome is finally reached. On Brazil nut agar the growth is very similar to that on cornmeal agar, but with a halo 1 to 2 mm. in width, showing the presence of an extracellular, proteolytic enzyme surrounding the thallus. The color of the spore mass at maturity is from Orange-Cinnamon to Mikado-Brown. On nut plugs the growth is rapid, and a gas with the odor of carbon bisulphide is evident. The color of the spore mass is Primrose-Yellow at first, Honey-Yellow to Tawny-Olive at maturity. At the end of two or three months all that remains of the nut plug is a mass of partially disintegrated cell walls in a mass of mycelium.

The growth on autoclaved rice is vigorous, with spore masses forming within forty-eight hours. There is little change in the color of the medium except for the development of a slight greenish-yellow tint below the spore mass. The color of the spore mass changes from Oil-Yellow to Orange-Cetrine. The odor of a 30-day old culture is very like that of cider vinegar. The nut strip above the water is entirely covered with spore masses within three days, but only about one-fourth of it is destroyed before it becomes too dry to support the fungus. A luxuriant growth of mycelium arises from the strip in the water, and usually the strip is destroyed before fifteen days.

TAXONOMY.—A culture of this species was sent to CHARLES THOM, and the following excerpt is taken from his reply dated January 29, 1920:

The organism belongs to the general group in which we are trying to separate three lines, the *Aspergillus oryzae-flavus* line, the *Aspergillus wentii* section, and the one which has been designated by KITA as *Aspergillus tamari*. This one, from the examination today, would appear to belong to the section containing *A. tamari*. Whether it is safe to identify it under an existing name or not would be doubtful.

5. BACTERIAL DECAY

GENERAL DESCRIPTION AND MORPHOLOGICAL CHARACTERS.—When Brazil nuts are affected by this bacterial decay, the shell is black and greasy, and usually exhales a rancid odor. When the shell is cracked open the remains of the kernel are found as a white mass which ordinarily fills only a small portion of the shell cavity. Microscopic examination of fragments of the refuse shows numerous bacterial spores, but usually no vegetative forms and no fungi. When dilution plates were made from the decayed residue, one spore-bearing organism largely predominated.

The vegetative cells of the organisms in cornmeal broth are rod-shaped, rounded at the ends, vigorously motile, and usually single but often in chains of from two to six individuals. The rods measure 2.5–5.0 $\mu \times 0.8$–1.2 μ. Spores are formed within forty-eight hours in one end of the vegetative cells. When the cells are stained by LOEFFLER'S method, the organism is found to have numerous long, peritrichiate flagella; stained with LOEFFLER'S methylene blue the protoplasm is seen to be granular with from two to four darkened patches which are unevenly distributed, usually giving a banded effect, although often the bands are oblique as well as horizontal (fig. 38). The organism stains readily with methylene blue, Gentian violet, and carbol-fuchsin, but it is Gram negative.

When sterile nut plugs were inoculated with the bacillus from pure culture, they were reduced in about fifteen days to an oily mass which, in all essential characters, was like the remains of the nut kernels in the natural cases of nut decay. Dilution plates made from nut meats that had decayed, following pure culture

inoculation, showed only one type of colony, and this proved to consist of the organism with which the plugs had been inoculated. The organism had no appreciable effect on the nut strip above the water, and the strip in the water was only very slowly decomposed, but strips in cornmeal bouillon were completely destroyed within ten to fifteen days. The organism grows best in the presence of air, as the colonies on all plated media and stab culture show, but deep lenticular colonies (fig. 40), and colonies next to the glass (fig. 39) in agar plates, as well as the faint line of growth along the stab, indicate that it is a facultative anaerobe.

While none of the usual tests for particular enzymes was made, the reactions in different culture media indicate the production of diastase, invertase, rennet, and pepsin. In Brazil nut agar plates there is formed a transparent halo about the colony, and as the opacity of the agar is due to the presence of solid proteid matter (**20**), the halo results from the digesting of these proteids. There is an abundant secretion of the protease which makes the halo, as the diameter of the transparent area is from two to three times that of the colony itself. This enzyme was precipitated as already described, and drops of a water solution of the dried precipitate placed on Brazil nut agar plates. A transparent area as large as the drop of solution was formed in a plate 2 mm. thick in from two to three hours.

The organism seems to be an undescribed one, and a complete description of it will be given in a separate paper.

6. Actinomyces decay

General description and morphology.—Empty shells that are intact and still retain their normal color are occasionally found among Brazil nuts. When these shells are cracked open a characteristic musty odor is evident, and the inner shell wall is seen to be covered with pinkish velvety pustules that are from one to several millimeters in diameter. Water mounts of pieces of a pustule show tenuous, mycelial-like strands, or chains of spores which readily stain with carbol-fuchsin. The filaments are not long but branch, and the mass is so bound together by the branches that it is quite impossible to separate entire filaments from the mass.

The filaments are never entirely straight nor yet very crooked, and chains of spores are usually contained in the free ends (fig. 37). No spirals were found on any of the media. The diameter of the filaments varies from 1.0 to 1.3 μ, and the oblong spores measure 1.6×0.8 μ.

The germination of spores was studied with an oil immersion lens, in a hanging drop prepared as follows. A thin film of synthetic agar was spread on a thin cover-glass, and a loop full of a dilute spore suspension placed on the agar film. This was inverted over a dry Van Tieghem cell. The water soon evaporated, leaving the spores in contact with the agar, where their germination was easily studied and camera lucida drawings made. According to DRECHSLER (**9**), *Actinomyces* spores produce from one to four germ tubes, "the approximate number being more or less characteristic of the species." This species produces one and two germ tubes which often branch directly on leaving the conidium (fig. 36).

The organism was studied in the manner suggested by CONN (**3**) and WAKSMAN (**33**), and the media were made in accordance with directions given by WAKSMAN (**33**). The following culture characters were noted:

CULTURAL CHARACTERS.—1. Synthetic agar: room temperature, after ten days: growth densely compact but thalli small, at first white, but after ten days Pale Pinkish Buff; aerial mycelium white and dense; soluble pigment none.

2. Calcium malate-glycerin agar: growth spreading and not zonated, bordered by submerged mycelial bands of varying width, pearl white; aerial mycelium short, loose, and pearl white; soluble pigment.

3. Glucose agar: growth luxuriant, color same as in synthetic agar, thallus conspicuously zonated; aerial mycelium white to Pale Pinkish Buff, powdery; soluble pigment none.

4. Glycerin agar: growth densely compact, not zonated, Pale Pinkish Buff; aerial mycelium powdery, white; soluble pigment none.

5. Brazil nut agar: growth rapid, densely compact with wide margin of submerged mycelium, white to Pale Pinkish Buff;

aerial mycelium dense, white; soluble pigment none; enzymatic zone three to four times the diameter of thallus.

6. Cornmeal agar: growth dense but zonated, Pale Pinkish Buff; aerial mycelium powdery; soluble pigment none.

7. Egg albumin agar: growth thin, conspicuously zonated, Pale Pinkish Buff; aerial mycelium powdery, Pale Pinkish Buff; soluble pigment none.

8. Nut plugs: growth vigorous, Pale Pinkish Buff; aerial mycelium powdery, white; medium not completely destroyed, but much shrunken and blackened.

9. Autoclaved rice: growth vigorous, Pale Pinkish Buff; aerial mycelium 2 cm.; almost completely destroyed in sixty days.

10. Potato plugs: growth vigorous, crumpled, Pale Pinkish Buff; aerial mycelium abundant, at first white, later Pale Pinkish Buff; medium with no change in color, much reduced in size in two months.

11. Carrot plugs: growth at first slow, appearing after five days, crumpled and dense, Pale Pinkish Buff; aerial mycelium powdery, at first white, later Pale Pinkish Buff; medium darkened near the growth, no change in color in other regions, much shrunken.

12. Brazil nut bouillon: growth surface pellicle, snow-white; aerial mycelium white, powdery; medium somewhat clarified.

13. Nut strips: growth slight on strip above water, and none on strip in water; no growth on surface of water.

BIOCHEMICAL FEATURES.—The proteolytic enzyme which makes the halo in Brazil nut agar plates was the only one studied, but the growth reactions in different media were taken to indicate the probable production of several other enzymes, diastase and invertase especially. The proteolytic enzyme was isolated by precipitation, as previously described, and its proteolytic power tested by placing drops of a water solution of the dried precipitate on Brazil nut agar plates. Transparent areas the size of the drops developed in from two to three hours, depending upon the thickness of the agar plates.

TAXONOMY.—The organism is an *Actinomyces* which, according to WAKSMAN'S key, belongs in division *B*, "no soluble pigment produced on gelatin or other protein media," and in section *I*,

"species strongly proteolytic; gelatin liquefied rapidly, milk clotted and peptonized rapidly." No species given in this division and section, however, has the characteristics of the one found in Brazil nut shells. It is therefore given the name of *Actinomyces brasiliensis*.

Actinomyces brasiliensis, n. sp.—Straight, branched hyphae 1.0–1.3 μ in diameter; spores borne in chains in free ends of hyphae, oblong, 1.6×0.8 μ; growth Pale Pinkish Buff on all agars except calcium malate-glycerin, on which it is white; zonated on glucose, cornmeal, and egg albumin agars; aerial mycelium on all media, white to Pale Pinkish Buff; no soluble pigment formed.

HABITAT.—Parasitic on kernels of Brazil nuts.

7. PHOMOPSIS DECAY

GENERAL DESCRIPTION.—Only one nut was found affected with *Phomopsis* decay, but because of its striking diagnostic features the fungus was isolated and studied. There was no external indication of the diseased condition, but the kernel of the nut was rich brown, with a few black specks near one end. The odor of the nut was pleasant and the taste agreeable. Stained hand sections showed that the mycelium of the fungus had penetrated into the radicle to considerable depth.

MORPHOLOGY.—The mycelium was tenuous, septate, and at first hyaline, but soon became brown or smoke colored. According to DIEDICKE (**8**), the form of the pycnidia is greatly varied. In the Brazil nut species several of the forms pictured by DIEDICKE were observed, but the one most commonly met with was mammiform, with a wartlike protuberance. The irregular pycnidial cavity so common to the genus was frequently observed, but a regular cavity was the rule. Two forms of spores were present in all pycnidia examined (fig. 50), and as is customary, the *Phoma* type will be designated as *A*, the filamentous as *B* spores. The *B* form did not germinate in hanging drop, a fact supporting the statement made by GROVE (**10**) that these may or may not be spores. When they fail to germinate they are probably what SACCARDO (**25**) took them to be, conidiophores, which according to GROVE "become more curved than when *in situ*." The *A*

spores are oblong-elliptical, hyaline, guttulate, and measure 5–7×1.7–3.5 μ. The *B* spores are filiform, usually hook-shaped, hyaline, continuous, and measure from 17–24.5×2–3.5 μ.

CULTURE CHARACTERS.—The fungus grew well on all media used. On cornmeal agar the thallus was circular and without zonations. A loose aerial mycelium covered the entire thallus, and numerous pycnidia varying in size were scattered over the surface of the plate. The pycnidia appeared simultaneously with the brown color, which was usually noticed after five or six days. On Brazil nut agar the growth was similar to that on cornmeal agar. The clear halo formed in the agar plate barely exceeded the size of the thallus. On autoclaved rice a brown or smoky color, due to the mycelial growth, was noticeable, but no color change occurred in the medium. Nut plugs were soon covered with a brown mycelium which later became almost black. The surface was soon covered with black, wartlike pycnidia, and the entire mass when cut through suggested a dried sponge. The odor was similar to that of very rancid nuts. The fungus made no growth on nut strips above the water, but a dense mass of mycelium, filled with black strands, developed on the strips in the water. These strips retained their form, but the cessation of mycelial growth, which occurred between ten and fifteen days after inoculation, marked the time of nutrient exhaustion.

TAXONOMY.—The fungus is a typical *Phomopsis* which approaches *P. aucubicola* Grove more nearly than any other described species. *A* spores are shorter, and this, coupled with the fact that it occurs on an unrelated host, necessitates describing it as new.

Phomopsis bertholletianum, n. sp.—Pycnidium dark brown, mammiform, with wartlike protuberance, irregular in shape and size, varying from 0.1 to 1.0 μ; conidiophores filiform, hyaline, continuous, 15–20 μ long, often indistinguishable from *B* spores. *A* and *B* spores present, *A* spores oblong-elliptical, hyaline, guttulate, 5–7×1.7–3.5 μ; *B* spores filiform, usually hook-shaped, hyaline, continuous, 17–24.5×2–3.5 μ.

HABITAT.—Parasitic on kernels of Brazil nuts.

8. Bitter rot

Figure 4 shows a part of a Brazil nut affected with bitter rot, and fig. 49 shows spores of the fungus, two of which have conidiophores attached. Neither the spores nor the mycelium was viable, and time prevented more than a superficial examination being made. The fungus is apparently a *Myxosporium*.

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EXPLANATION OF PLATES VIII–XII

All drawings were made with camera lucida.

PLATE VIII

FIG. 4.—Part of Brazil nut kernel affected by bitter rot; ×1.

FIG. 5.—Thalli of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×$\frac{3}{4}$.

FIG. 6.—Thallus of *Actinomyces brasiliensis* n. sp. on cornmeal agar; ×2½.

FIG. 7.—Colony of *Bacillus* from Brazil nut on cornmeal agar; ×2.

FIG. 8.—*Pellioniella macrospora* n. sp. in autoclaved rice; tube on right 20 days old; tube on left 10 days old; ×1.

FIG. 9.—Brazil nut 30 days after inoculation with *Pellioniella macrospora* n. sp.; nut plug at top marks place of inoculation, and along two edges inner seed coat removed to expose blackened endosperm; ×1.

FIG. 10.—Thallus of *Actinomyces brasiliensis* n. sp. on Brazil nut agar, surrounded by transparent area in which proteids have been digested owing to secretion of proteolytic enzyme; ×1½.

FIG. 11.—Thallus of *Pellioniella macrospora* n. sp. on cornmeal agar: three zones: (1) scarcely visible outer zone of white; (2) zone of nearly same width that is green in growing thallus; (3) inner black circle; ×¾.

FIG. 12.—*Actinomyces brasiliensis* n. sp. on potato plug after 10 days; ×1.

PLATE IX

FIG. 13.—Paraphyses, immature conidia, and conidiophores of *Pellioniella macrospora* n. sp.; ×500.

FIG. 14.—Conidia-like cells of hyphae of *P. macrospora* n. sp. from diseased tissue of Brazil nut; ×500.

FIG. 15.—Mature, immature, and transitional stages in development of conidia of *P. macrospora* n. sp.; ×500.

FIG. 16.—Germination of immature conidia of *P. macrospora* n. sp., planted in same hanging drop with mature conidia shown in figure 18; ×500.

FIG. 17.—Germination of mature conidia of *P. macrospora* n. sp.; germ tubes from one to two hours longer in emerging than those of immature conidia shown in figure 17; ×500.

FIG. 18.—Hypha of *P. macrospora* n. sp., showing most common type of cell; ×500.

FIG. 19.—Hypha of *P. macrospora* n. sp. from near pycnidium; ×500.

FIG. 20.—Conidia-like cells of hyphae of *P. macrospora* n. sp., taken from culture in autoclaved rice; ×500.

FIG. 21.—Section of pycnidium of *P. macrospora* n. sp., enlarged about 450 diameters.

FIG. 22.—Hyphae of *P. macrospora* n. sp., showing two types of cells; ×500.

PLATE X

FIG. 23.—Conidia of *Cephalosporium bertholletianum* n. sp.; ×500.

FIG. 24.—Hyphae, conidiophores, and spore masses surrounded by water drops, *C. bertholletianum* n. sp.; ×500.

FIG. 25.—Germinating conidia of *C. bertholletianum* n. sp., two hours after planting; ×500.

FIG. 26.—Germinating conidia of *C. bertholletianum* n. sp., twenty hours after planting; ×500.

FIG. 27.—Hyphae of Brazil nut *Aspergillus* bearing sterigmata; ×500.

FIG. 28.—Hypha of Brazil nut *Aspergillus* bearing short stalked conidiophores; ×500.

FIG. 29.—Conidia of Brazil nut *Aspergillus;* ×500.

FIG. 30.—Mature conidiophores of Brazil nut *Aspergillus;* ×500.

FIG. 31.—Hyphae showing branching habit and anastomosis, Brazil nut *Aspergillus;* ×500.

FIG. 32.—Early stages of conidiophores of Brazil nut *Aspergillus;* ×500.

PLATE XI

FIG. 33.—Microtome section of normal nut kernel showing tissues named in order, beginning at top: endosperm, epidermis, cortex, procambium, and medulla; ×500.

FIG. 34.—Microtome section of Brazil nut affected by *Pellioniella macrospora* n. sp., showing dense mycelial growth in endosperm region; ×1000.

FIG. 35.—Microtome section of Brazil nut affected by *P. macrospora* n. sp., showing relation of fungus to host tissues; ×500.

FIG. 36.—Germinating conidia of *Actinomyces brasiliensis* n. sp.; ×1000.

FIG. 37.—Hyphae and conidia, *Acinomyces brasiliensis* n. sp.; ×1000.

FIG. 38.—Vegetative cells of Brazil nut bacillus; ×1000.

FIG. 39.—Colony of Brazil nut bacillus growing near glass in cornmeal agar plate; ×50.

FIG. 40.—Deep colony of Brazil nut bacillus n. sp.; ×50.

FIG. 41.—Surface colony of Brazil nut bacillus n. sp.; ×50.

PLATE XII

FIGS. 42–49, and 51 are of a *Fusarium* which causes dry rot of Brazil nuts.

FIG. 42.—Conidial variation and anastomosis; ×500.

FIG. 43.—Typical hyphae showing difference in size and branching habit; ×500.

FIG. 44.—Hyphae bearing single conidiophores and conidia anastomosed to hyphal cells by germ tube; ×500.

FIG. 45.—Terminal chlamydospores; ×500.

FIG. 46.—Germinating conidia in hanging drop culture; ×500.

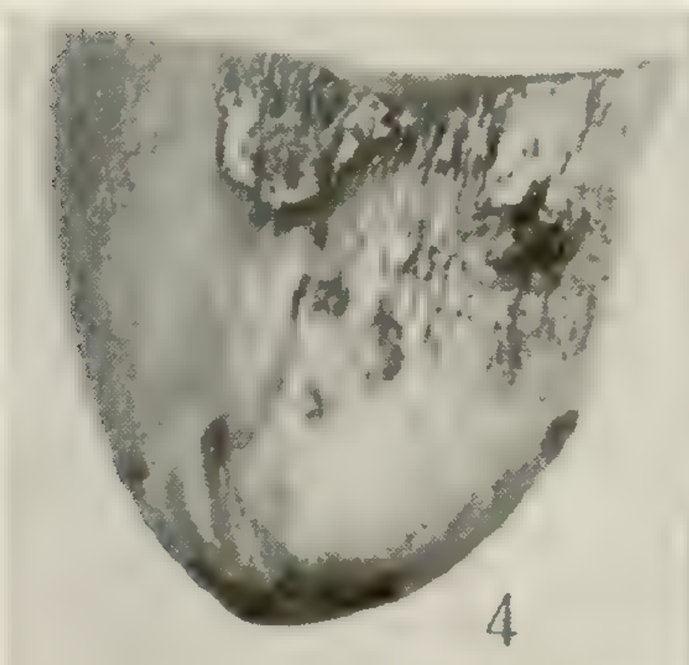
FIG. 47.—Anastomosing conidia and hyphae from culture plates; ×500.

FIG. 48.—Anastomosis of germinating conidia in hanging drop; ×500.

FIG. 49.—Conidia and conidiophores of bitter rot fungus, taken from pustules on diseased kernel; ×500.

FIG. 50.—*Phomopsis* conidia, two having germ tubes attached; ×500.

FIG. 51.—Hyphae of *Fusarium* from washed agar plates; ×500.



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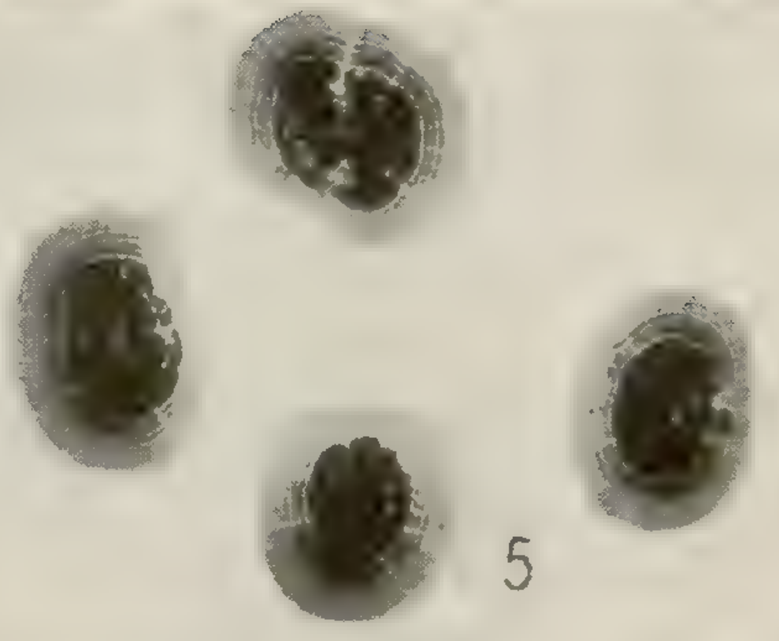

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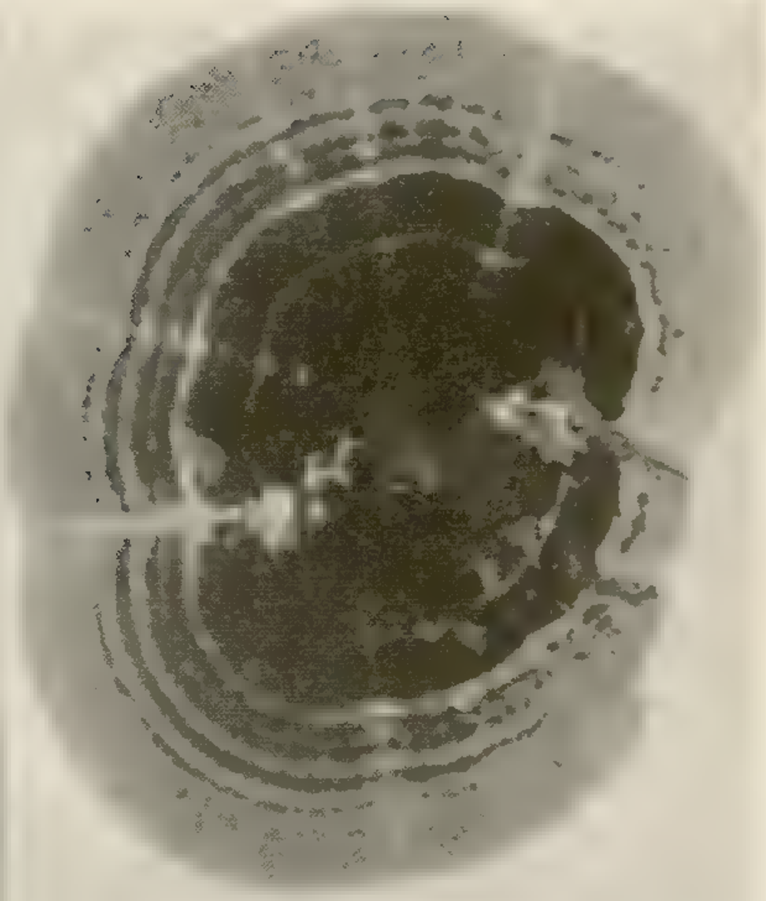

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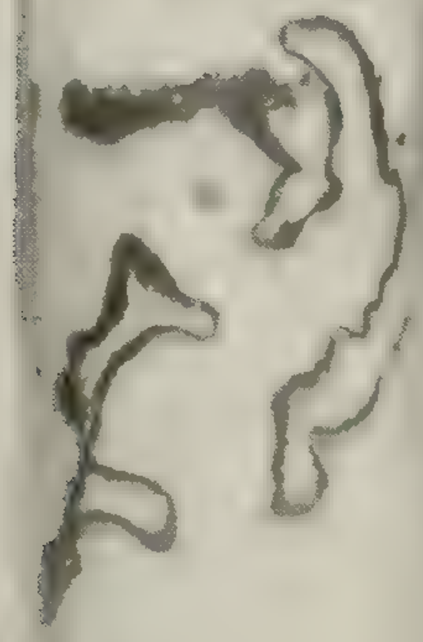

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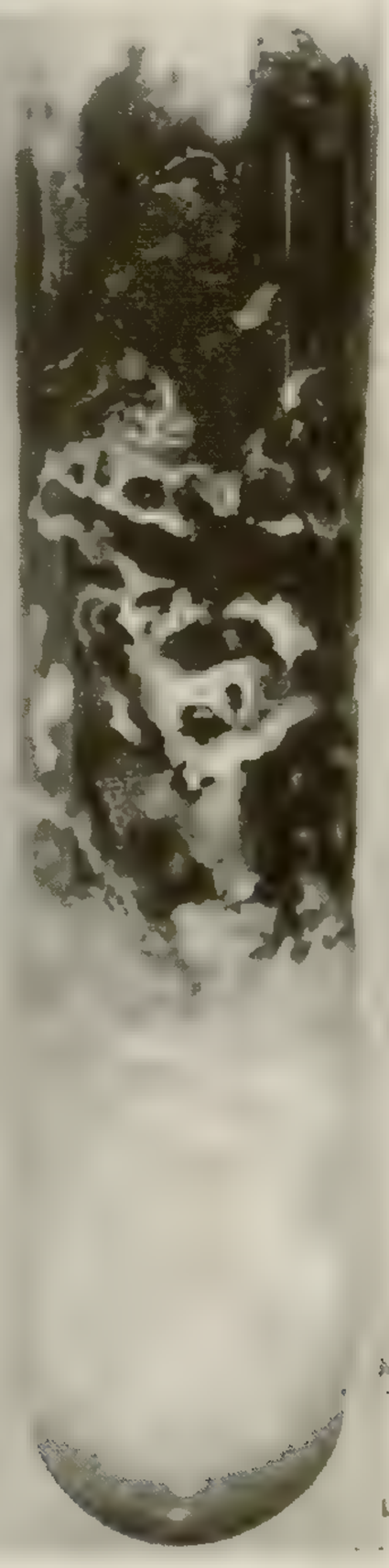

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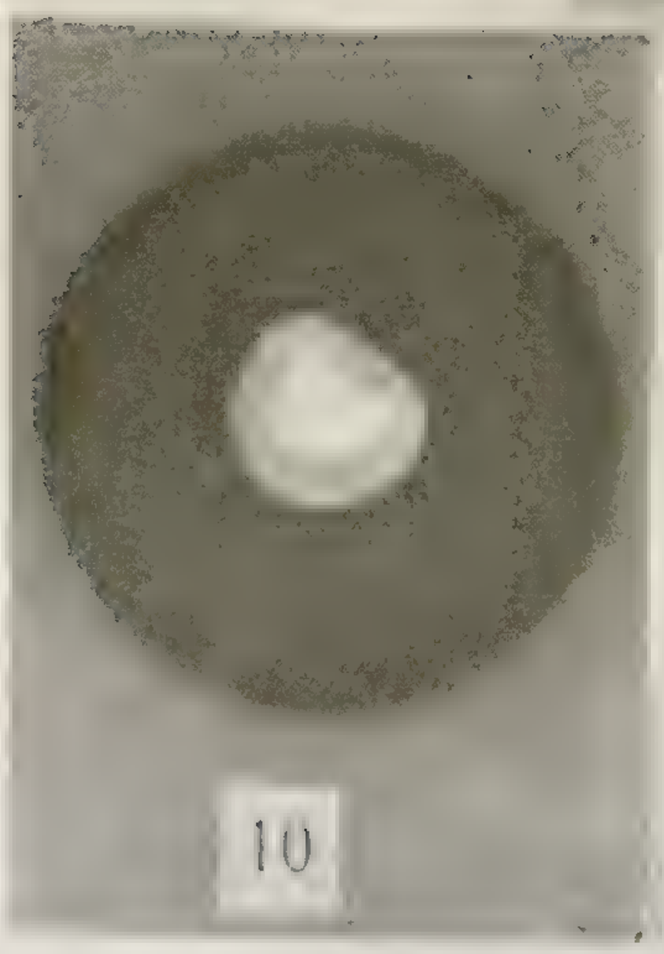

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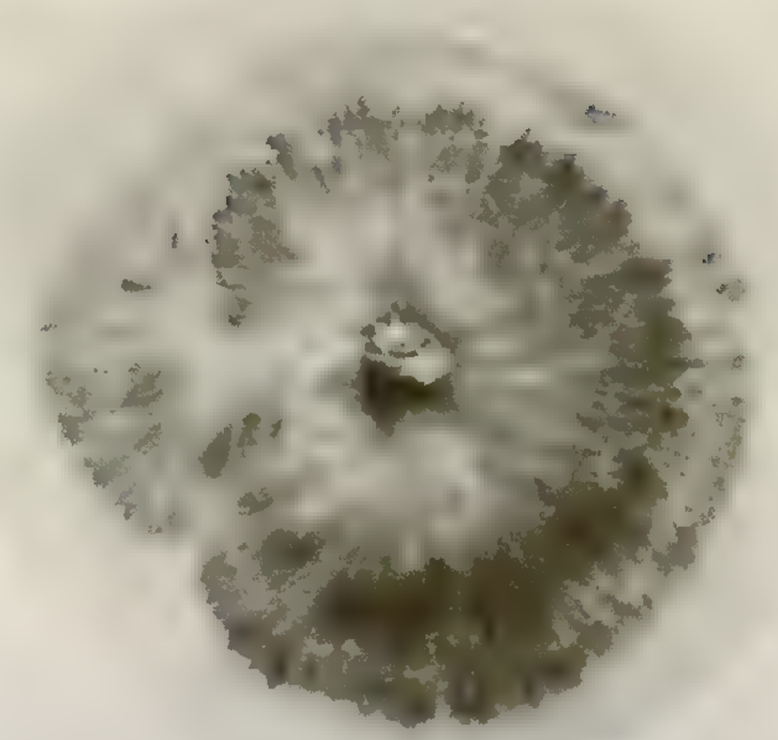

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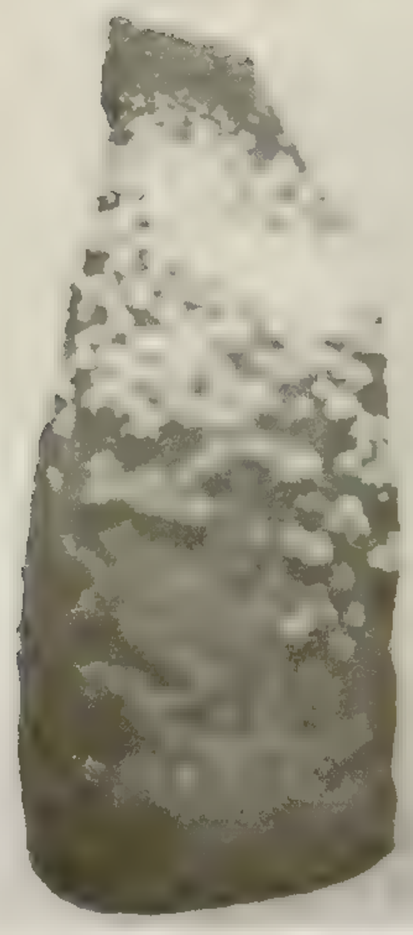

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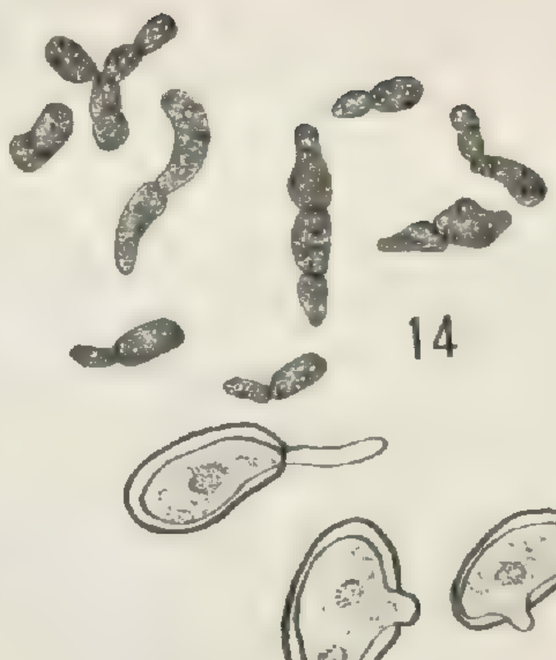

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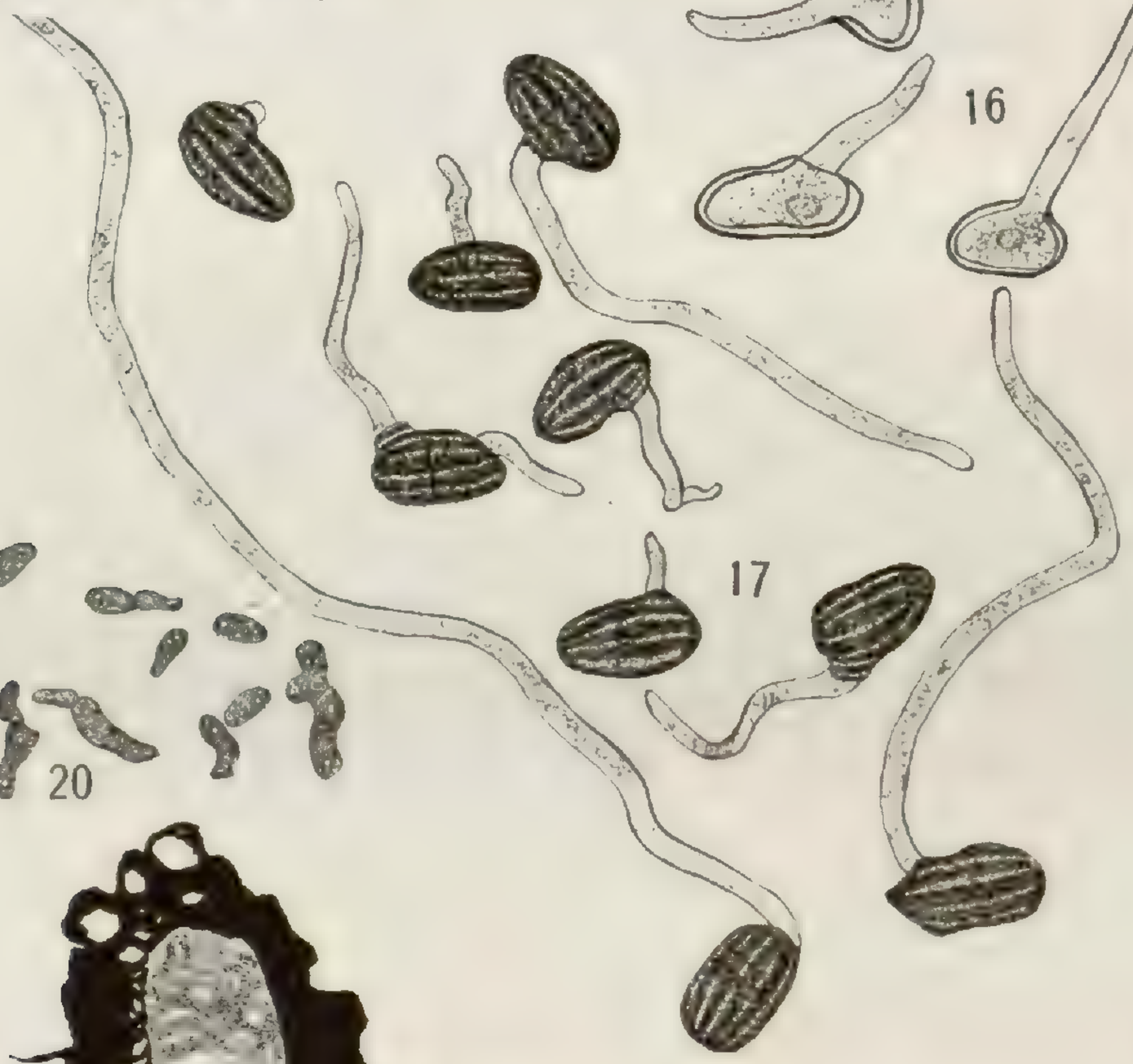

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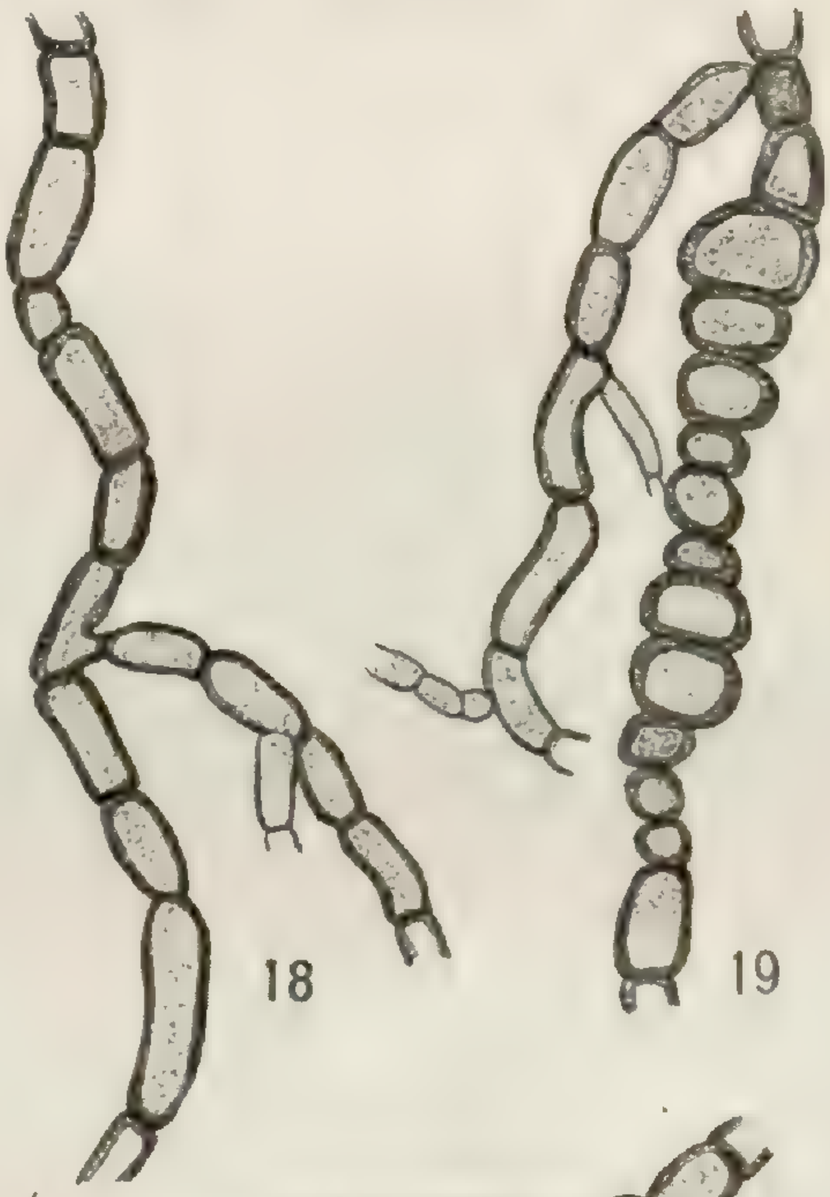

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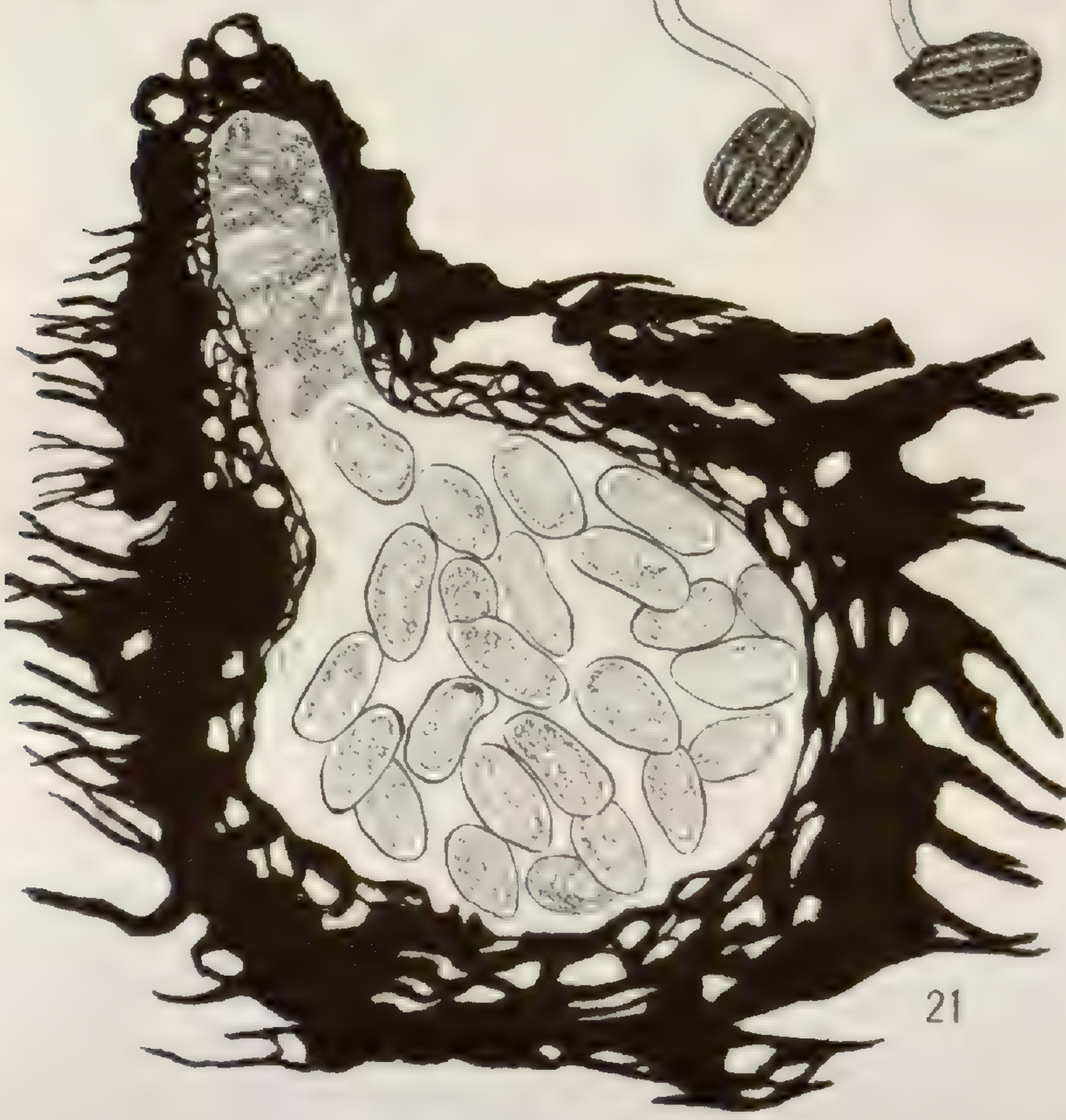

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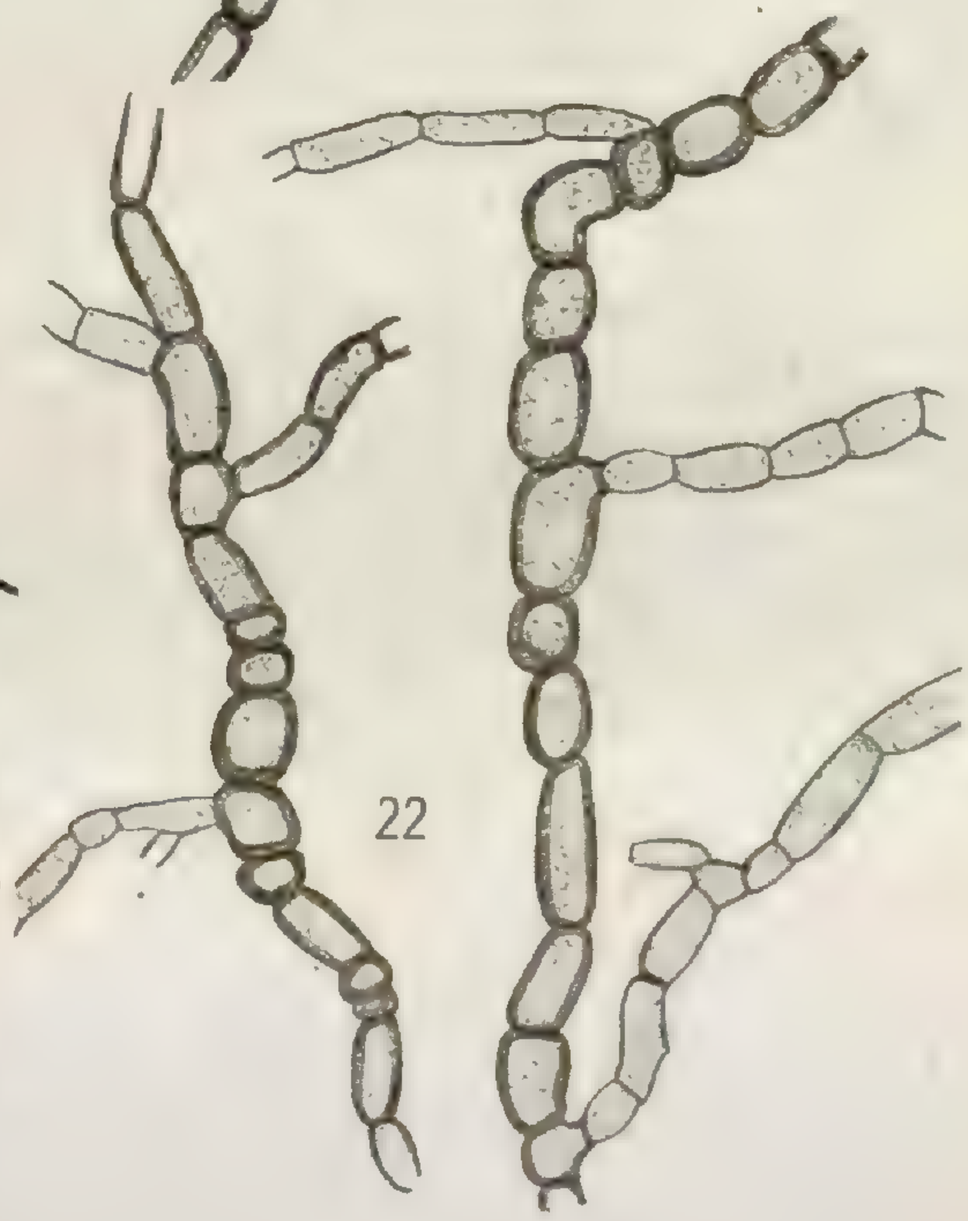

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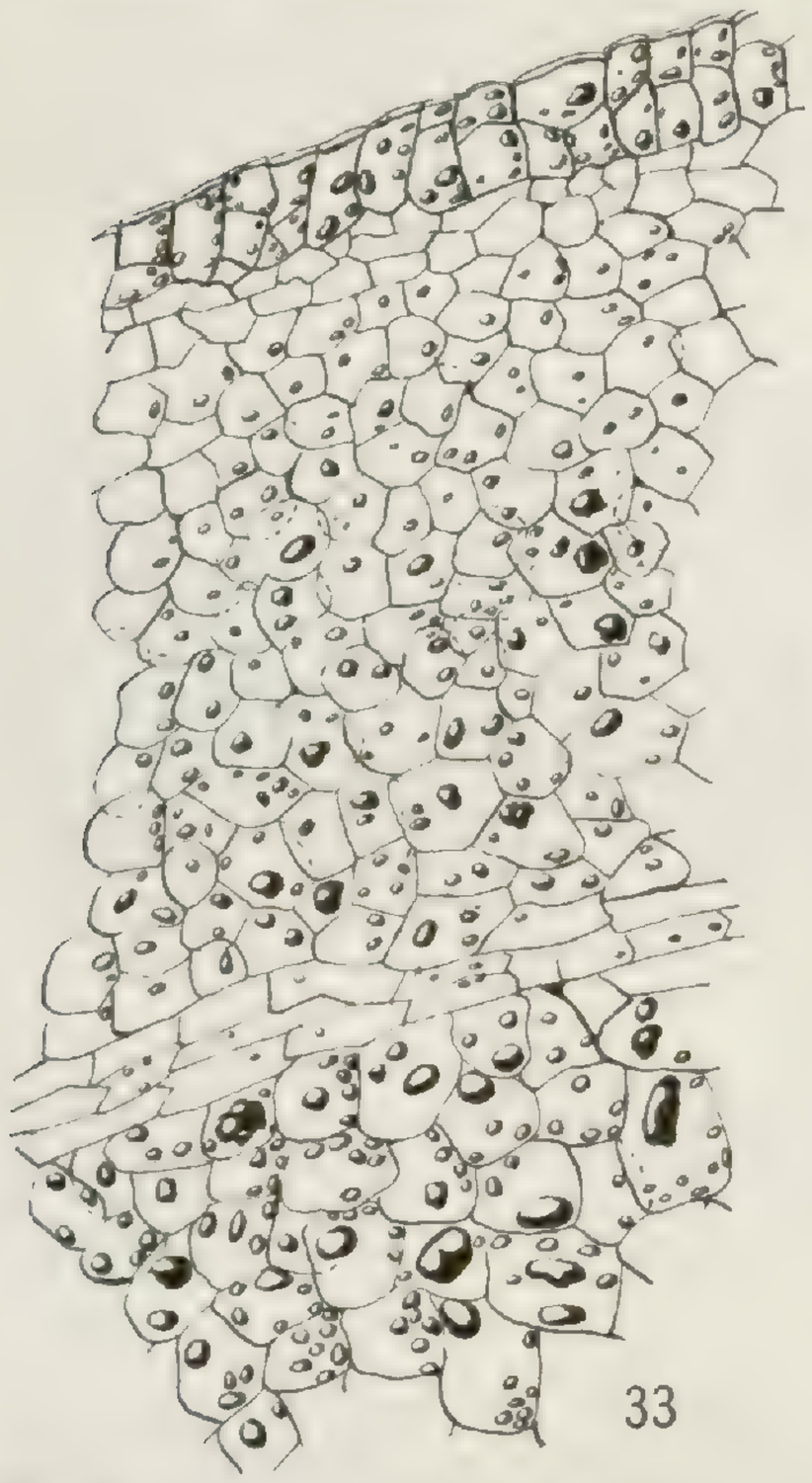

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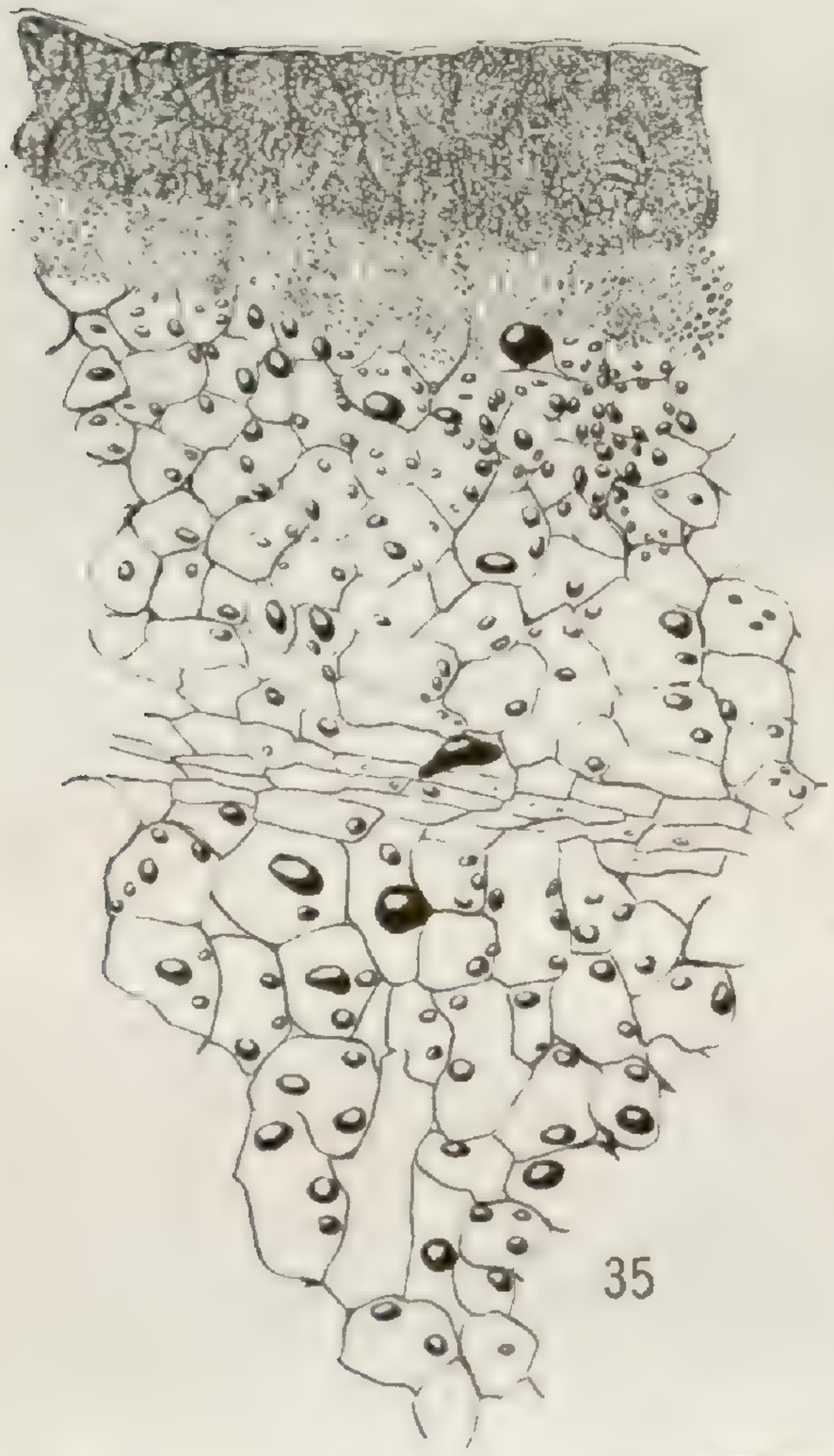

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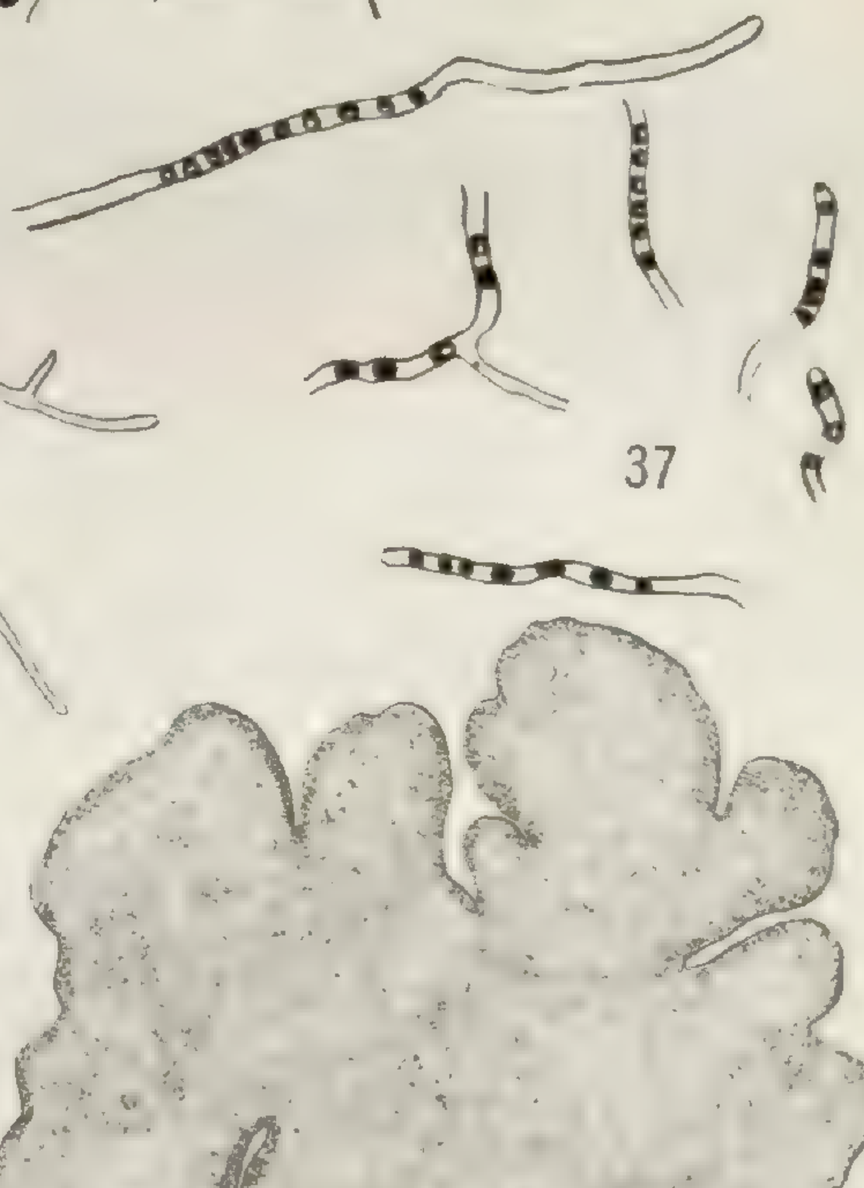

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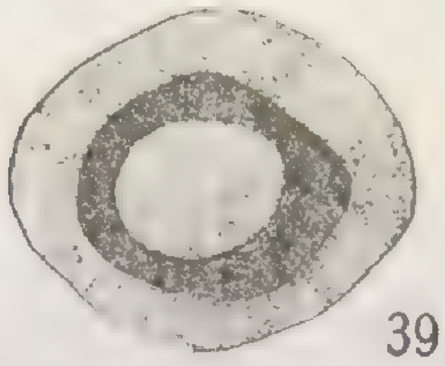

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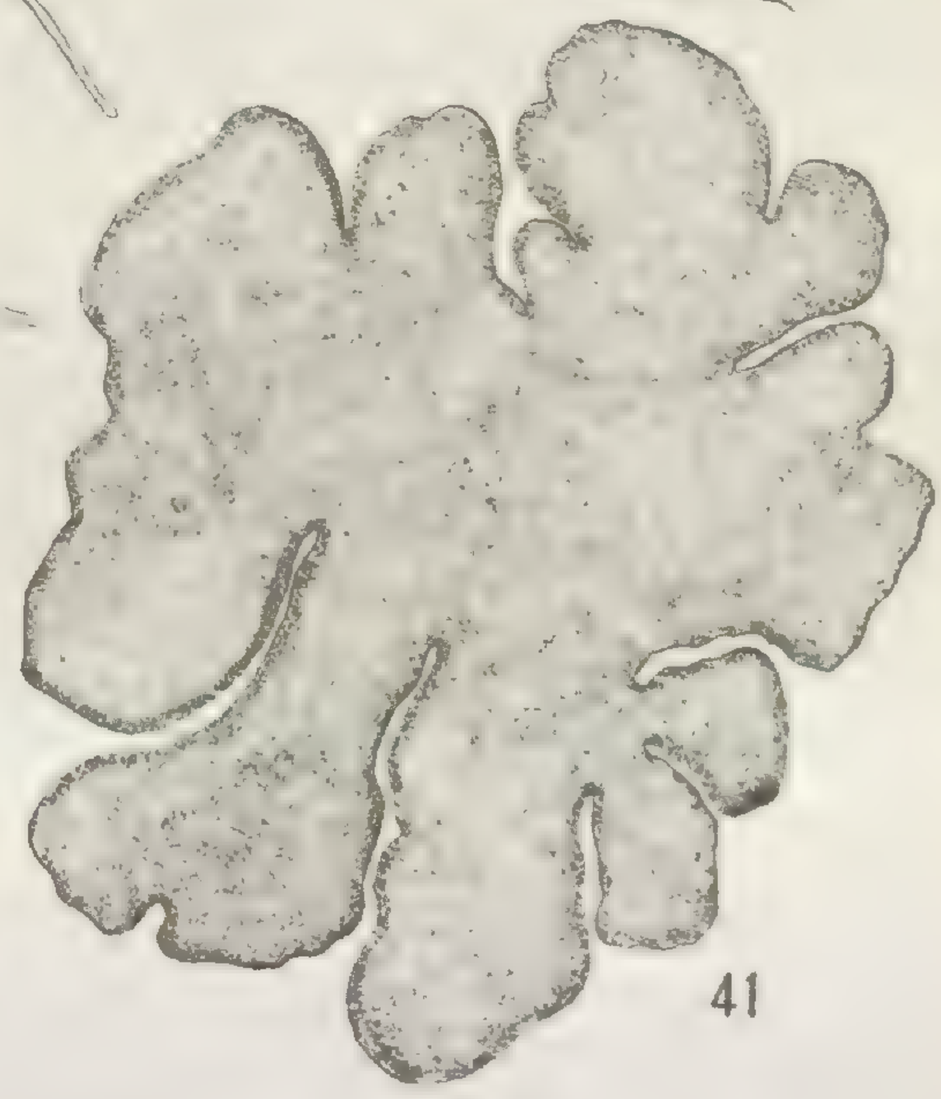

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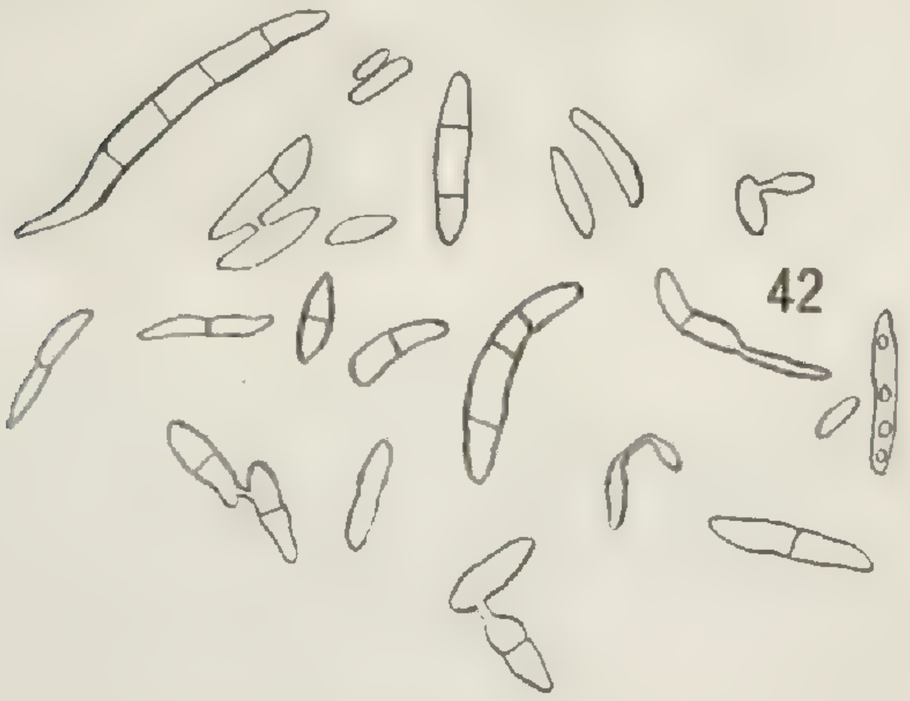
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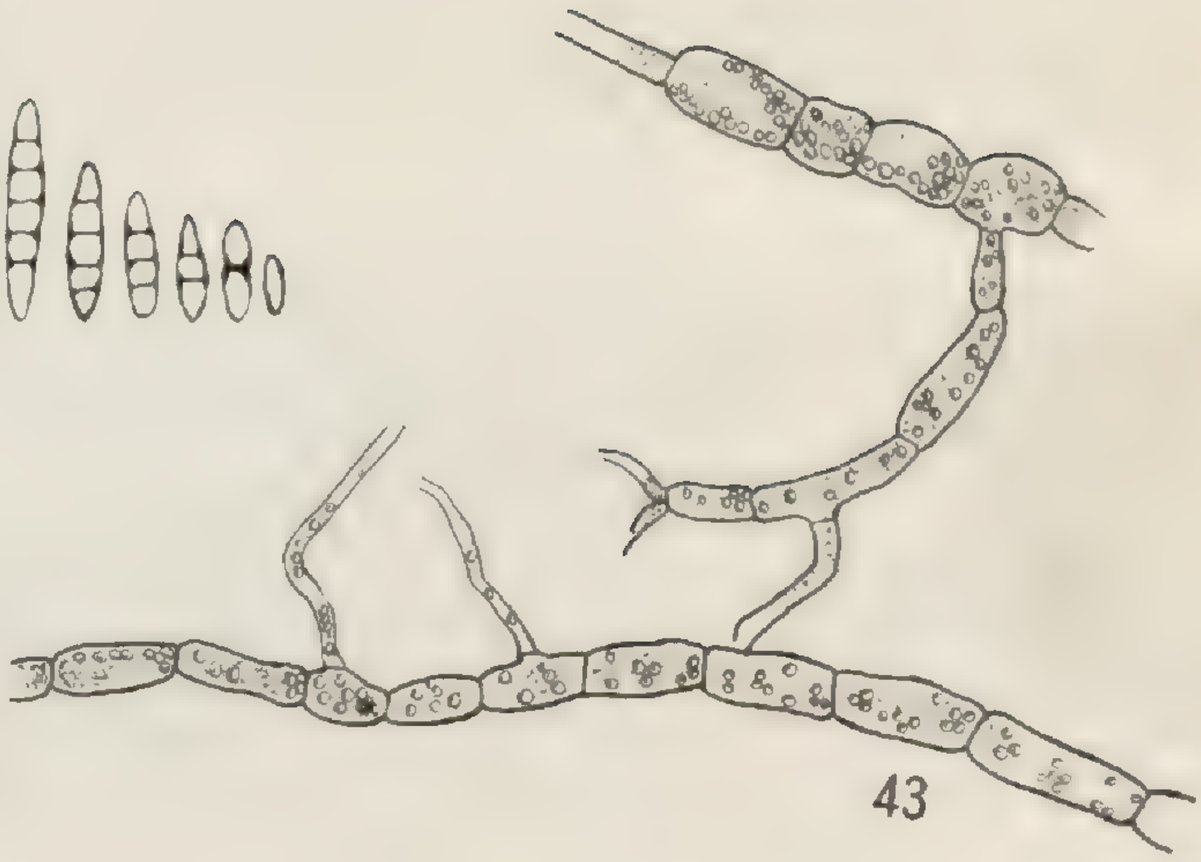
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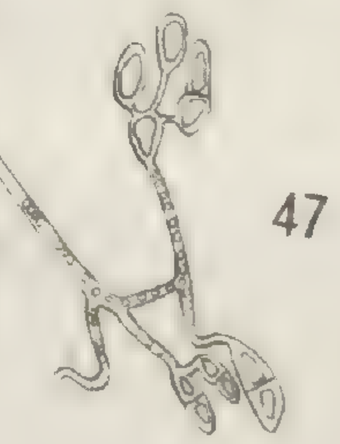
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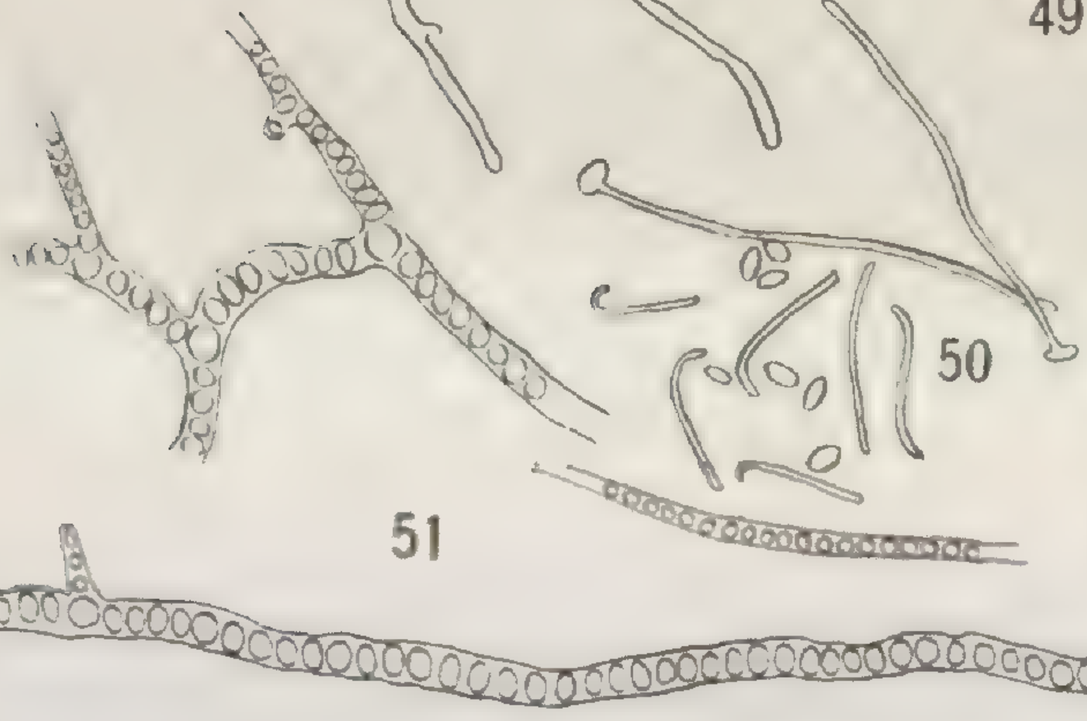
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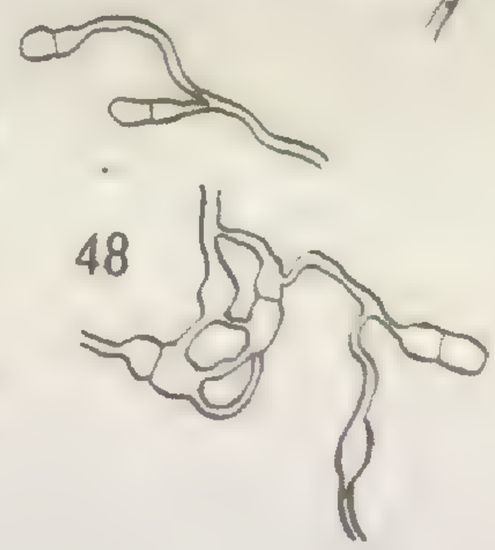
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GROWTH RINGS IN A MONOCOTYL

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 285

CHARLES J. CHAMBERLAIN

(WITH SIXTEEN FIGURES)

The principal object of this paper is to announce the discovery of growth rings in a monocotyl, but some observations upon growth rings in other plants may not be out of place. The most familiar example of periodic growth is seen in the annual rings of Gymnosperms and dicotyls; but even when there is a strong tendency to form only one ring every year, there are numerous variations, especially when the rings are very wide.

In *Melia azedarach* the annual rings are often more than a centimeter in width, but it is common to find in each season's growth a dozen or more secondary rings which are easily seen with the naked eye. The part of the ring formed in the spring and summer is quite sharply differentiated from that formed in the autumn, and it is in this autumn wood that the secondary rings are most conspicuous. In the spring and summer wood the few rather indefinite secondary rings are due to varying proportions of tracheae and tracheids. The tracheae of summer wood are not very different from those of early spring; while in the autumn wood the larger cells merely start to develop into tracheae. They have transverse walls, which in some cases begin to break down, but here the development ends. The tracheids of the autumn wood are numerous and very thick-walled, so that this part of the ring is extremely hard. It is evident that the secondary rings are due to periodic acceleration and retardation of growth, which causes them to show some of the features characteristic of ordinary annual rings.

Casuarina tenuissima affords another instance of numerous rings. A shoot about 3 mm. in diameter and only a few weeks old showed five or six well marked rings, due to an alternation of tracheae and tracheids. The plant from which the shoot was taken was

growing in the greenhouse, and the number of rings corresponds, roughly, to the number of times the plant was watered thoroughly. Several years ago, in the neighborhood of Jalapa, Mexico, where it is rather rainy throughout the year, a species of *Piper* was noticed which showed no growth rings; while the same species, a few miles farther east, where there is a sharp alternation of wet and dry seasons, showed the anticipated rings. These are examples of immediate response to rather slight changes in conditions. At the other extreme are plants which show no response to seasonal conditions, but nevertheless are susceptible to stronger stimuli.

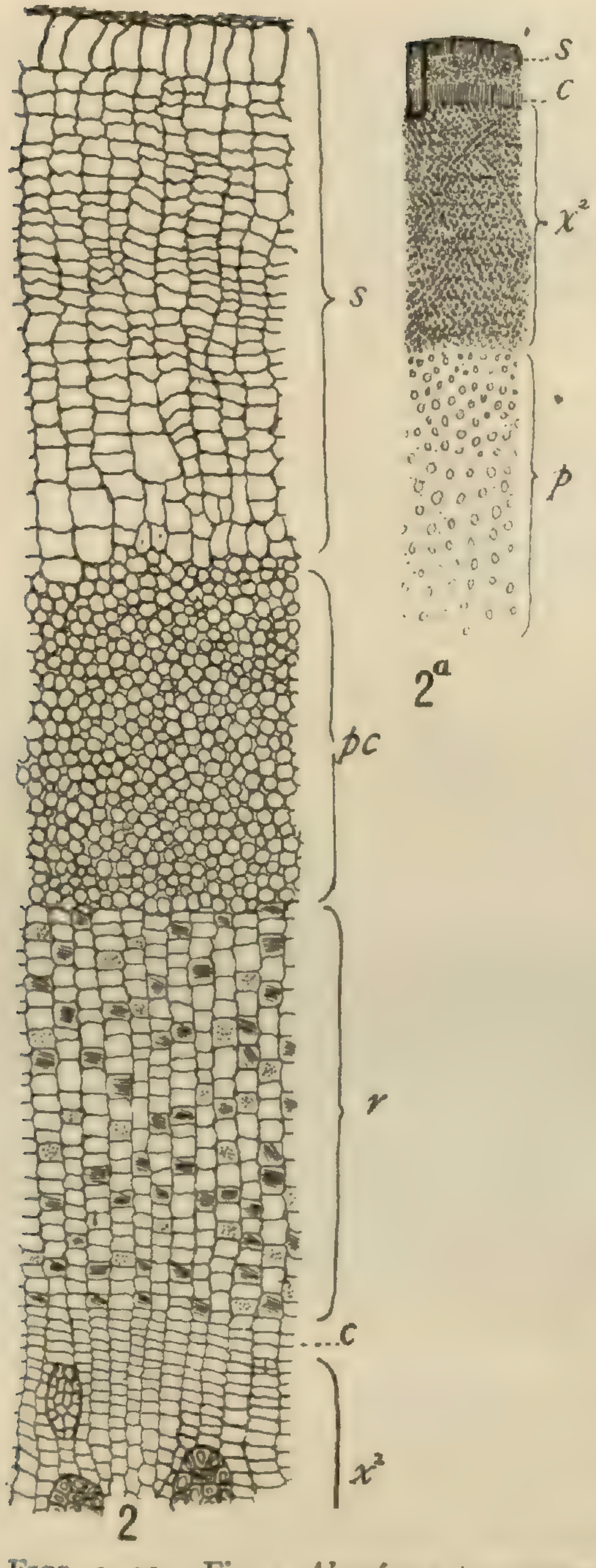
Interesting growth rings which do not mark the number of years, but correspond to longer intervals, are found in the cycads. *Dioon edule*, after a period of coning or after damage by fire, loses all its leaves and goes into a prolonged resting stage which may last for several years. When it resumes activity and produces a new crown, a vigorous growth of wood takes place, with the formation of large tracheids, which, following the small tracheids of the nearly exhausted condition, produce a ring having the characters of an ordinary annual ring in Gymnosperms. These prolonged resting periods occur at long intervals, so that the number of rings would be of slight value in estimating the age of a plant. A section of the trunk of a specimen of *D. edule* 1.5 to 2 m. in height would enable one to estimate the interval between successive growth rings, since the approximate age of the plant could be determined; but at present it is not easy to secure such a section. In *D. spinulosum* the rings look like those of *D. edule*, but a ring is produced with the formation of every crown of leaves. Since crowns in this species are usually formed every other year, the number of rings indicates about half the age of the plant. In these cases the ring is a response to a change in conditions, but a very decided change is necessary to produce the result.

It has long been known that some arborescent monocotyls, like *Dracaena*, *Yucca*, and *Aloe*, produce a distinct zone of secondary tissue surrounding the primary and derived from a meristematic region showing the characters of cambium. In 1912, while studying cycads in South Africa, I cut into a large plant of *Aloe ferox*

to get material for demonstration purposes, and it was surprising to find growth rings so conspicuous that they could be seen where the stem was cut with an ax. Pieces to show both primary and secondary structures were preserved in formalin, and later *Aloe pleuridens*, *A. ciliaris*, and *Dracaena Hookeriana* were collected for comparison.

FIG. 1.—*Aloe ferox* at Cathcart, South Africa, January 1912; about 3 m. in height.

Aloe ferox in the field presents a picturesque appearance, looking as if an *Agave* had developed a tall trunk (fig. 1). It is associated with other xerophytic plants as bizarre as itself, among them tree forms of *Euphorbia* more than a dozen meters in height, species of *Encephalartos*, and others not so large but equally peculiar. Most of the material was collected near Grahamstown, South Africa, in January 1912, from a stem 15 cm. in diameter and about 3 m. high. In transverse section the zone of secondary xylem



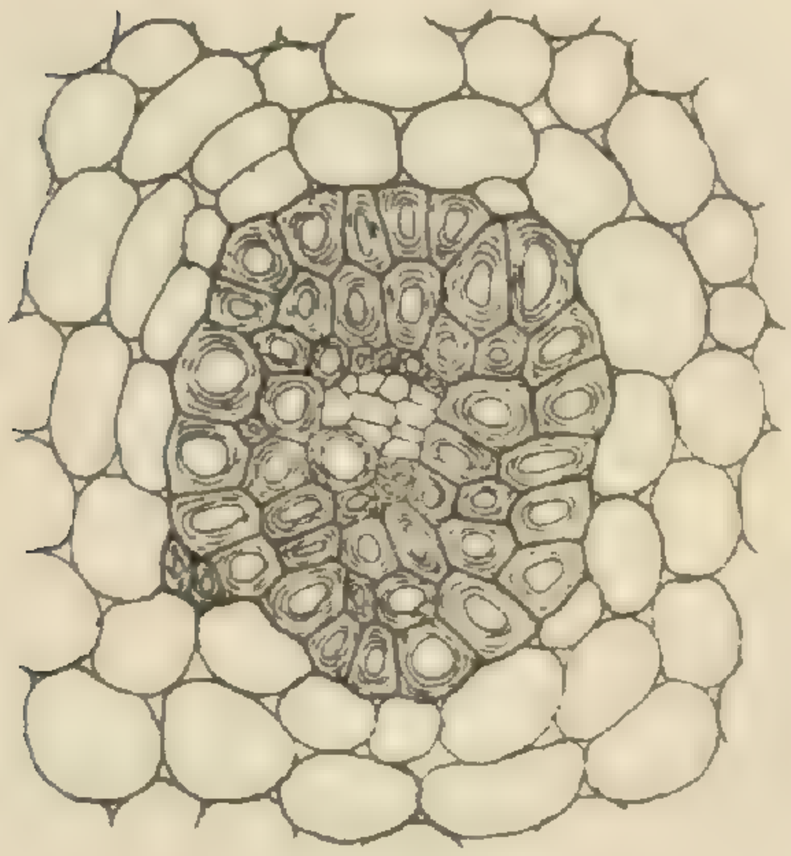
FIGS. 2, 2a.—Fig. 2, *Aloe ferox*, transverse section of part of stem: *s*, secondary cortex; *pc*, primary cortex; *r*, outer region of cells cut off by cambium and containing numerous raphides; *c*, cambium; x^2, region of secondary bundles from inner region of cells cut off by cambium; ×40; fig. 2a, *Aloe ferox:* part of transverse section of stem, natural size; fig. 2 shows part included in rectangle at upper left corner: *s*, secondary cortex; *c*, cambium; x^2, region of secondary bundles; *p*, primary polystelic region.

was 2 cm. and the cortex 4 mm. in width; so that the central region, nearly 10 cm. in diameter and consisting of primary structures, gave the whole section somewhat the appearance of a large pith surrounded by a narrow zone of wood and a scanty cortex.

The general topography of a small portion of a transverse section, natural size, is shown in fig. 2a. In the primary region (*p*) the bundles are large and scattered, as in a cornstalk; while in the zone of secondary growth (x^2) the vascular bundles are so regularly arranged, that to the naked eye they form a pattern like the chasing on a watch. The cambium (*c*), which is giving rise to secondary bundles, the secondary cortex (*s*), and some of the primary cortex between these two zones of secondary growth, are also visible to the naked eye.

The phellogen, with the secondary cortex produced by it, the inner cambium with its derivatives, and also the primary cortex (*pc*) between the two secondary products, are shown in fig. 2. The walls of the secondary cortex are

slightly thickened, but thoroughly suberized. Beneath the secondary cortex is the primary, consisting of loose rounded cells with



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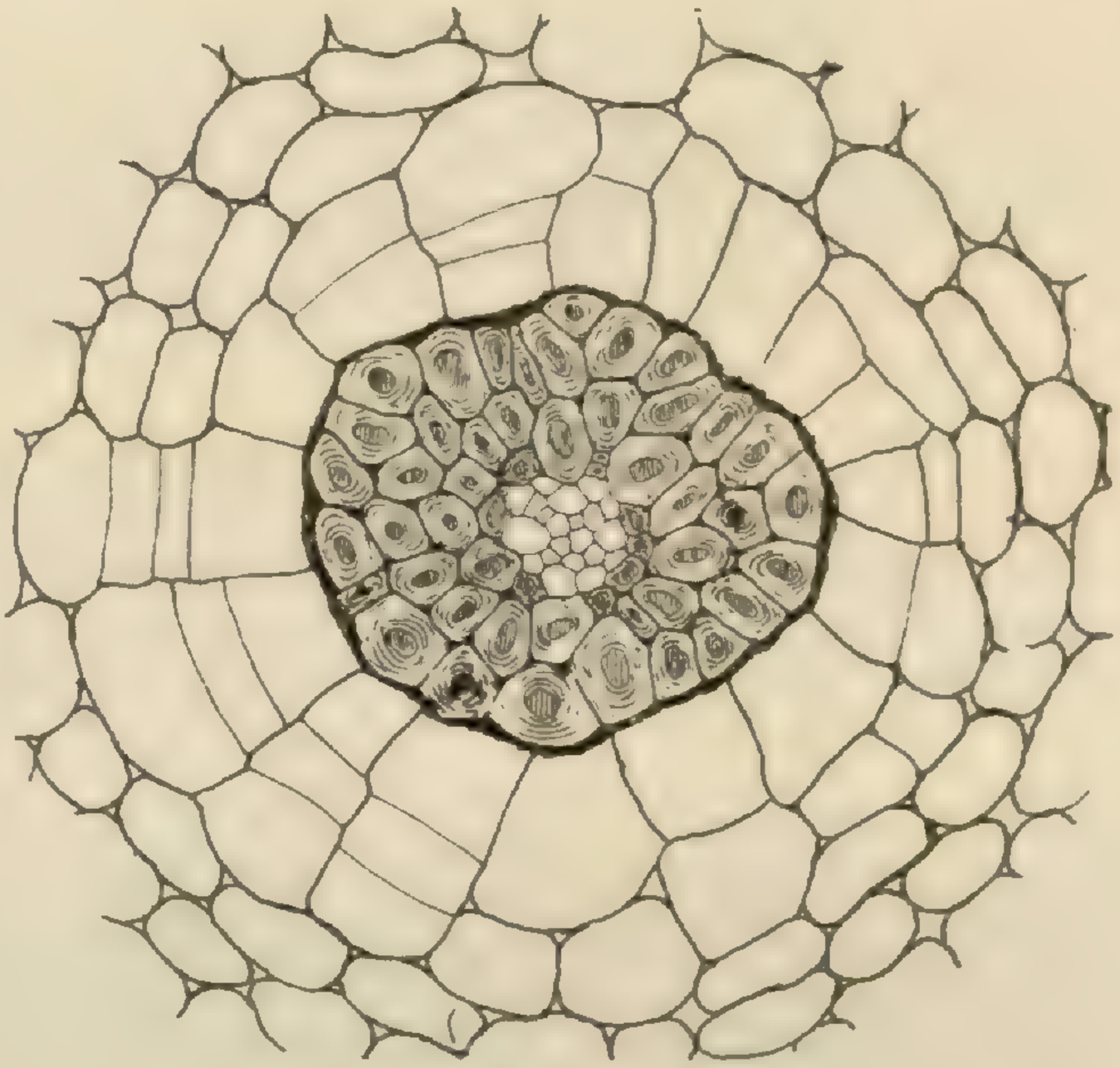

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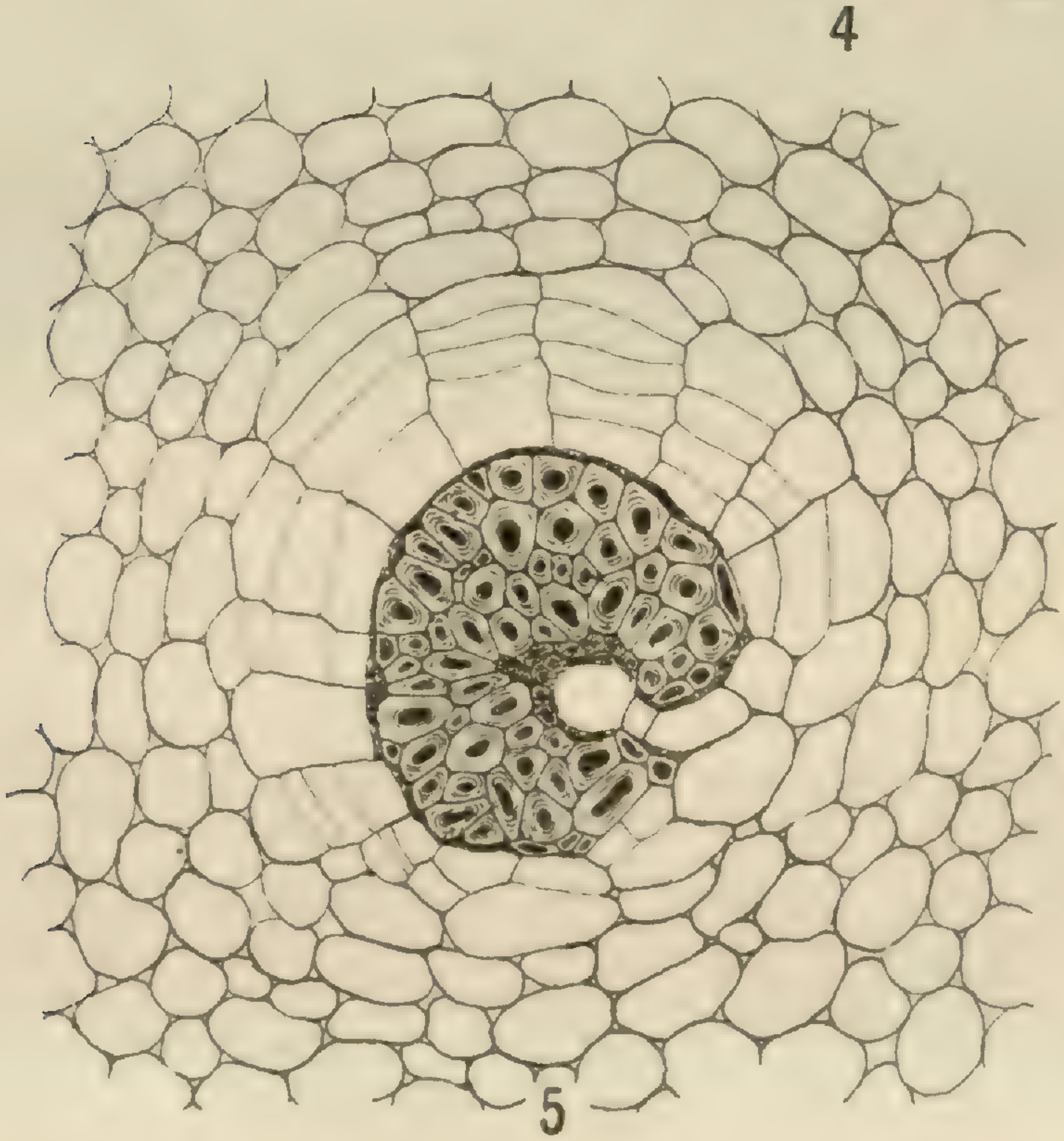

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FIGS. 3–5.—FIG. 3. *Aloe ferox:* normal bundle of primary polystelic region; ×100; fig. 4. *Aloe ferox:* bundle of primary polystelic region, showing clogged lumen of tracheids; some of cells immediately surrounding bundle becoming meristematic; ×100; fig. 5, *Aloe ferox:* more advanced condition than in fig. 4; ×100.

cellulose walls, and between the primary cortex and the primary polystelic region is the zone which contains the secondary vascular bundles and shows the growth rings.

The primary polystelic region, in transverse section, looks somewhat like an immense cornstalk, with large bundles toward the center and smaller ones at the outside; but the structure of the individual bundles is very different from that in corn, for the bundles in *Aloe* have no sheath and most of them are completely amphivasal. There seem to be two types of vascular bundles in

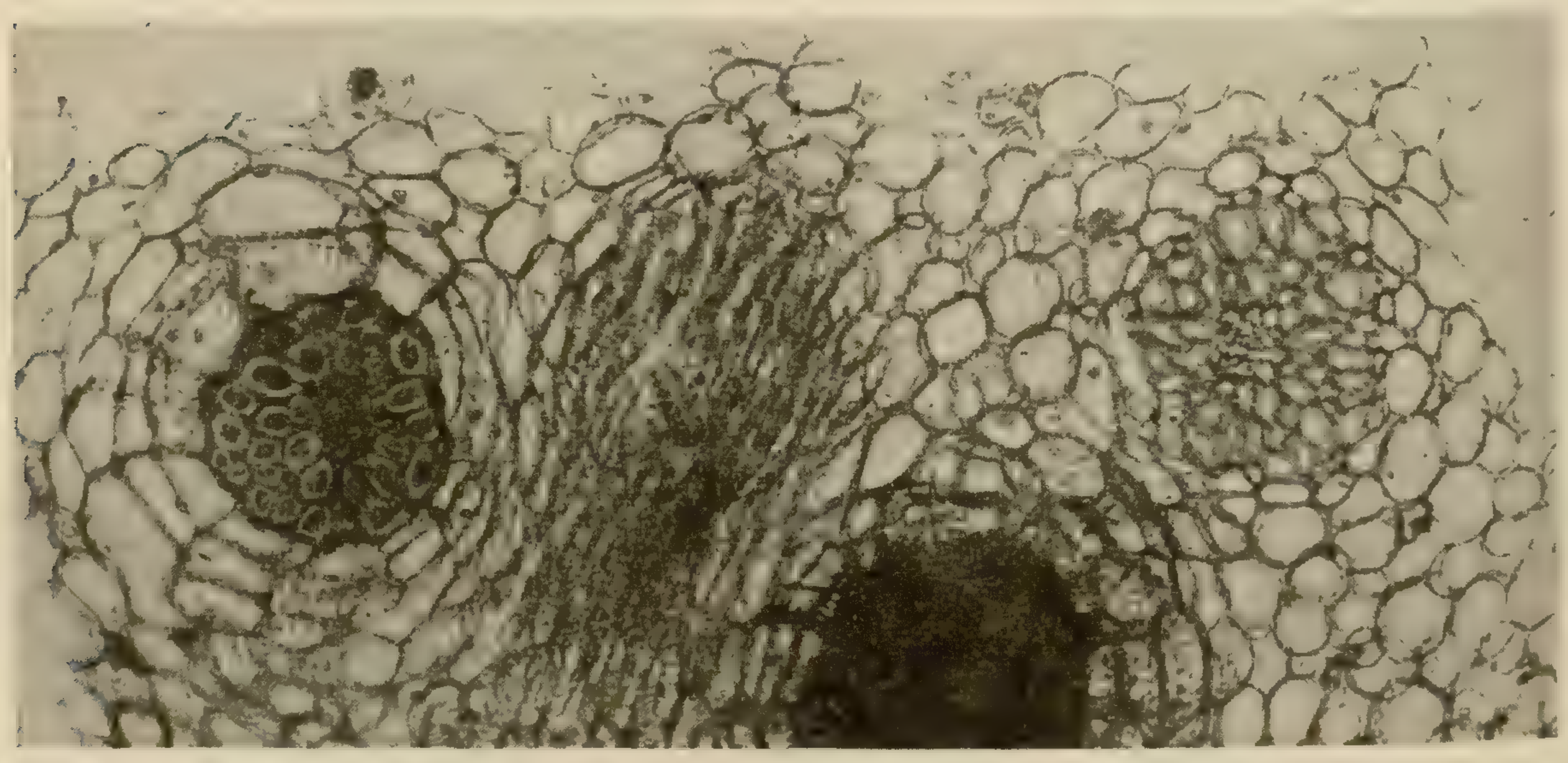
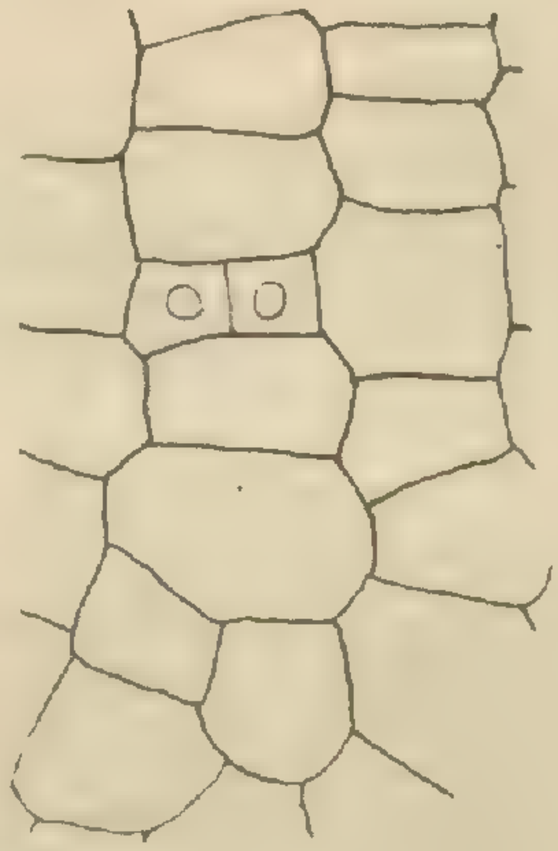


FIG. 6.—*Aloe ferox:* showing both types of primary bundles; ×100

this primary region. In one type the bundles have normal xylem and phloem, except that the phloem has very few companion cells (fig. 3). The other type is peculiar. The phloem begins to disorganize and finally disappears, while the lumen in both tracheids and vessels becomes clogged with the same material found in the disorganizing phloem. Some of this material can also be seen surrounding the bundle itself. An early stage, shown in fig. 4, and a later stage, shown in fig. 5, are characteristic. In the latter figure the contents of the xylem cells are much denser and the phloem cells have become almost indistinguishable, while the adjacent thin-walled parenchyma is crowding into the space left vacant by the disorganizing phloem.

Another peculiar feature of the bundle with disorganizing phloem is the appearance of vigorous meristematic activity in the cells surrounding the xylem. These cells behave like a cambium, so that rows consisting of as many as eight cells may be formed (figs. 4, 5). If differentiation should take place, we should expect to find a xylem zone and, perhaps, phloem surrounding the primary bundle; but development stops soon after the stage shown in fig. 5, before any lignification can be detected. The distribution and general appearance of the primary bundles are shown in fig. 6.

That the secondary growth in some monocotyls, like *Yucca*, *Dracaena*, and *Aloe*, results from meristematic activity is well known. The piece of a transverse section of *Aloe ferox*, natural size (fig. 2*a*), already referred to, and a somewhat magnified view of the origin of secondary structures (fig. 2), show the position of the structures to be described. The phellogen is evidently hypodermal in origin, and it builds up a limited amount of secondary cortex, with rectangular cells in regular rows abutting upon the smaller spherical cells of the primary cortex. The cambium which gives rise to the vascular structures is pericyclic in origin, and, as seen in transverse section, gives rise to long rows of cells. The cells on the outer side of the cambium undergo comparatively little differentiation; they enlarge to about twice the size of the cambium cells, and many of them become almost entirely filled with needle-shaped crystals of calcium



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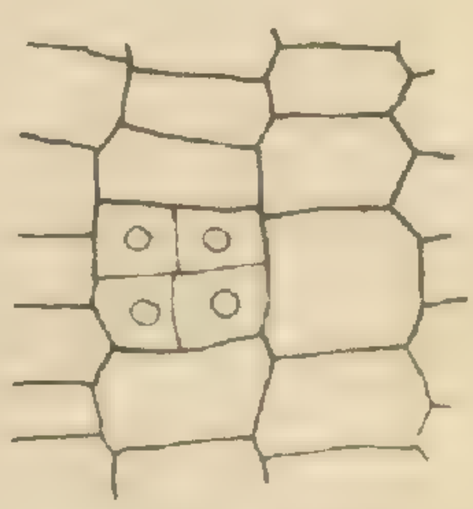

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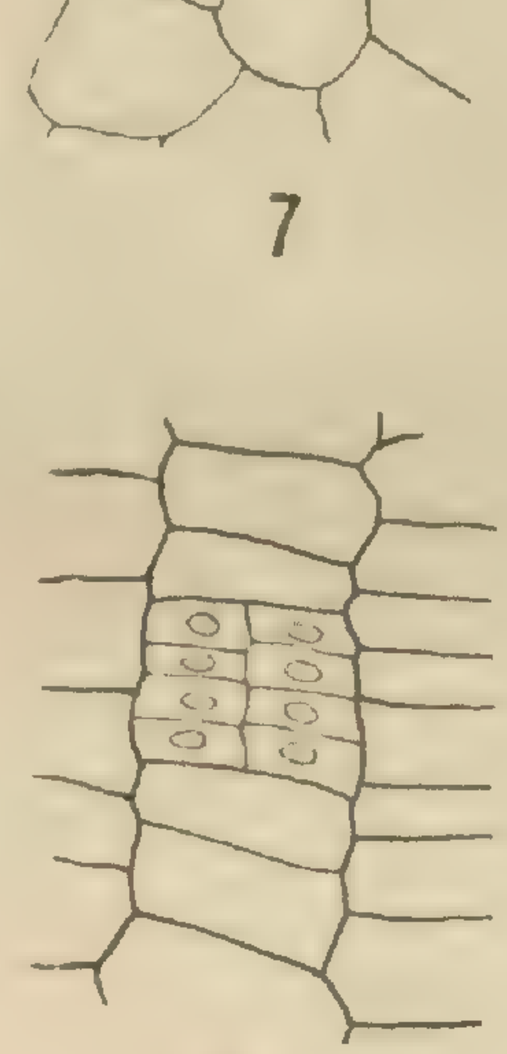

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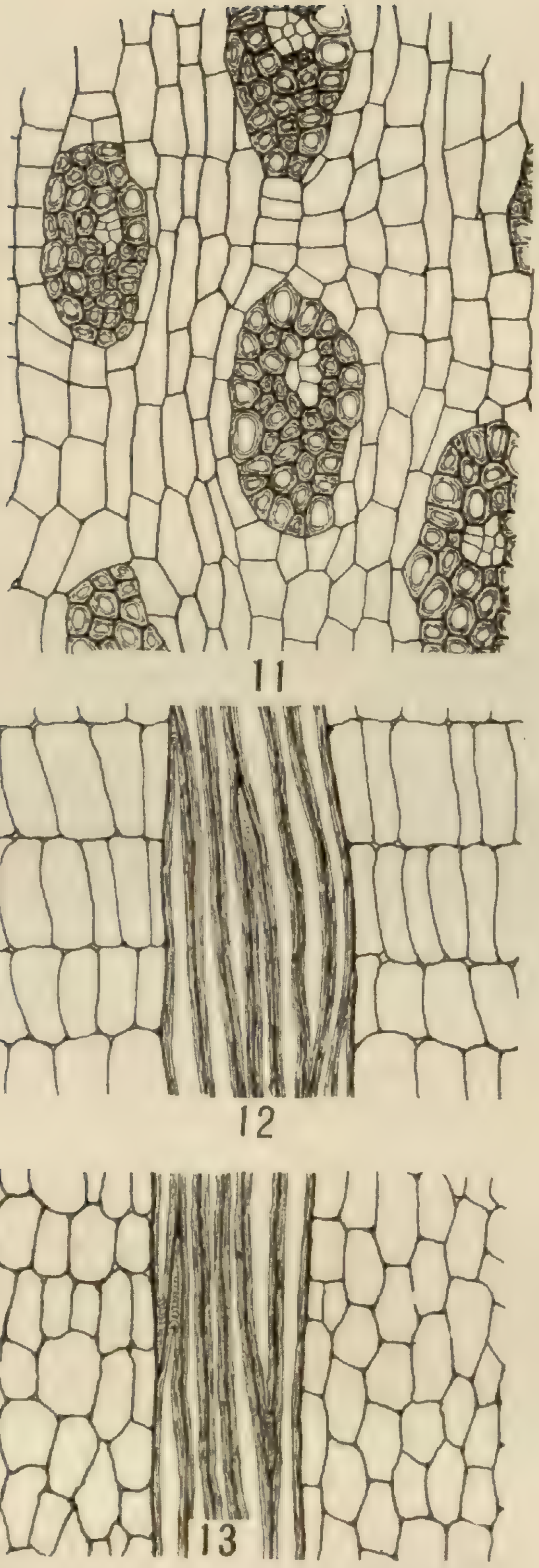
FIGS. 7–10.—*Aloe pleuridens:* four early stages in development of secondary bundle; ×100.



FIGS. 11–13.—*Aloe ferox:* fig. 11, transverse section of secondary bundle; fig. 12, longitudinal radial section; fig. 13, longitudinal tangential section; ×100.

oxalate; but the cell walls thicken very little and retain the cellulose reaction (fig. 2 *r*.)

The cells formed centripetally from the cambium give rise to the secondary woody structures which show the growth rings. The development of the bundle was not studied very thoroughly in *Aloe ferox*, but the early stages are about the same as in *A. pleuridens*. As seen in transverse section, a cell of the row produced by the cambium divides, the two resulting cells divide, and the process continues until forty or fifty cells are formed (figs. 7–10). Differentiation of the young cells of the vascular strand begins to take place before the full number of cells has been reached. These bundles are completely amphivasal and there is no sheath of thick-walled cells. The phloem is scanty and companion cells are rare. There is no degeneration or clogging of the lumen in the secondary bundles, and there is no meristematic activity in any of the surrounding cells, like that which characterizes many of the bundles of the primary cylinder. Since the

bundle is completely amphivasal, there is no cambium between the xylem and phloem, like that found in the primary bundles of many monocotyls (fig. 11).

The xylem consists almost entirely of tracheids with bordered pits and with walls so thick and hard that sectioning is difficult. The cells cut off from the inner side of the cambium and not taking part in the formation of the bundles keep, more or less perfectly, their linear arrangement. They are short, somewhat rectangular in radial view, and are arranged in very definite rows (fig. 12). The tangential arrangement is not so regular (fig. 13). While they thicken only a little, they become thoroughly lignified and extremely hard, so that they add to the difficulty of cutting sections. They are marked by numerous small simple pits.

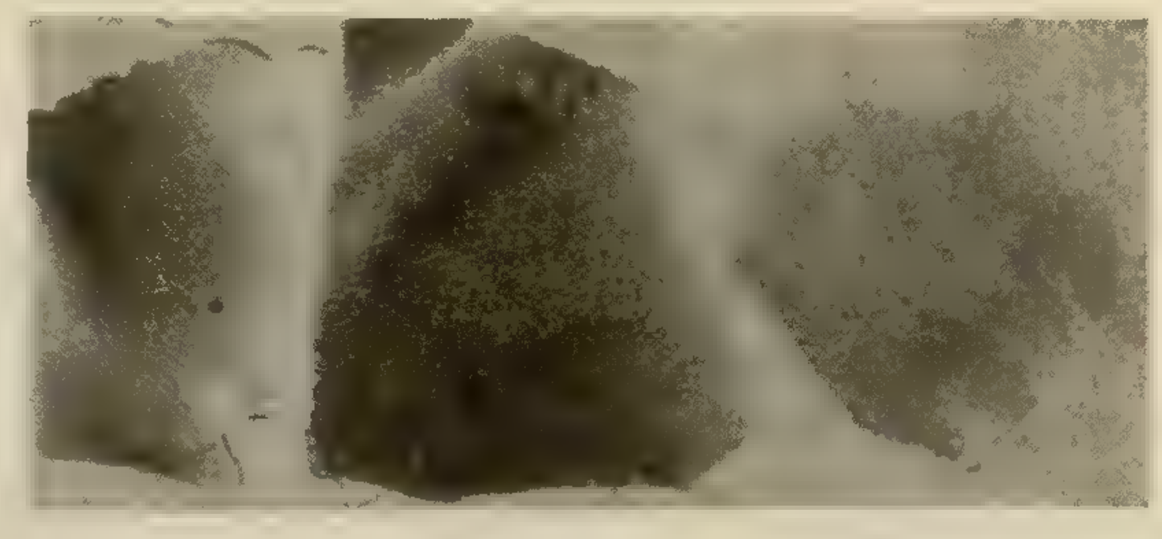


FIG. 14.—*Aloe ferox:* three thick transverse sections of stem; natural size.

The growth rings constitute the most striking feature of the stem. DE BARY, in his *Comparative anatomy of vegetative organs of the phanerogams and ferns*, remarked that, while there seemed to be no reason why growth rings should not be formed in woody monocotyls, none had ever been observed. An examination of the literature of vascular anatomy failed to yield any account of such rings; but, to make certain that nothing had been overlooked, I wrote to Professor JEFFREY, and he not only informed me that such rings had never been reported, but also gave some suggestions which greatly facilitated the investigation.

To the naked eye the growth rings are obvious, but under a 16 mm. objective no one would suspect their presence. In *Dioon*, where growth rings are obvious to the naked eye but not so conspicuous under the microscope, the rings are due to the fact that cells formed at the close of a growth period are somewhat smaller and have thicker walls than those formed when growth is resumed. In *Aloe ferox* the explanation is not so evident. An examination of fig. 14, showing three thick transverse sections, indicates that the rings can be seen, even in a half-tone reproduction.

The rings can be seen more clearly by looking across the figure from nearly the level of the paper. The negative was made twice the size of the section, and the illustration reduced to natural size. The same sections without reduction are shown in fig. 15. The appearance, under a low magnification, is shown in fig. 16, which includes six of the growth rings. The rings are not at all conspicuous, and the number of rings probably could not be counted in the illustration. Even with the position on the rings marked with the numerals 1–6, they are not easily identified. Two structural features cause a ring. At the close of the growing period a few

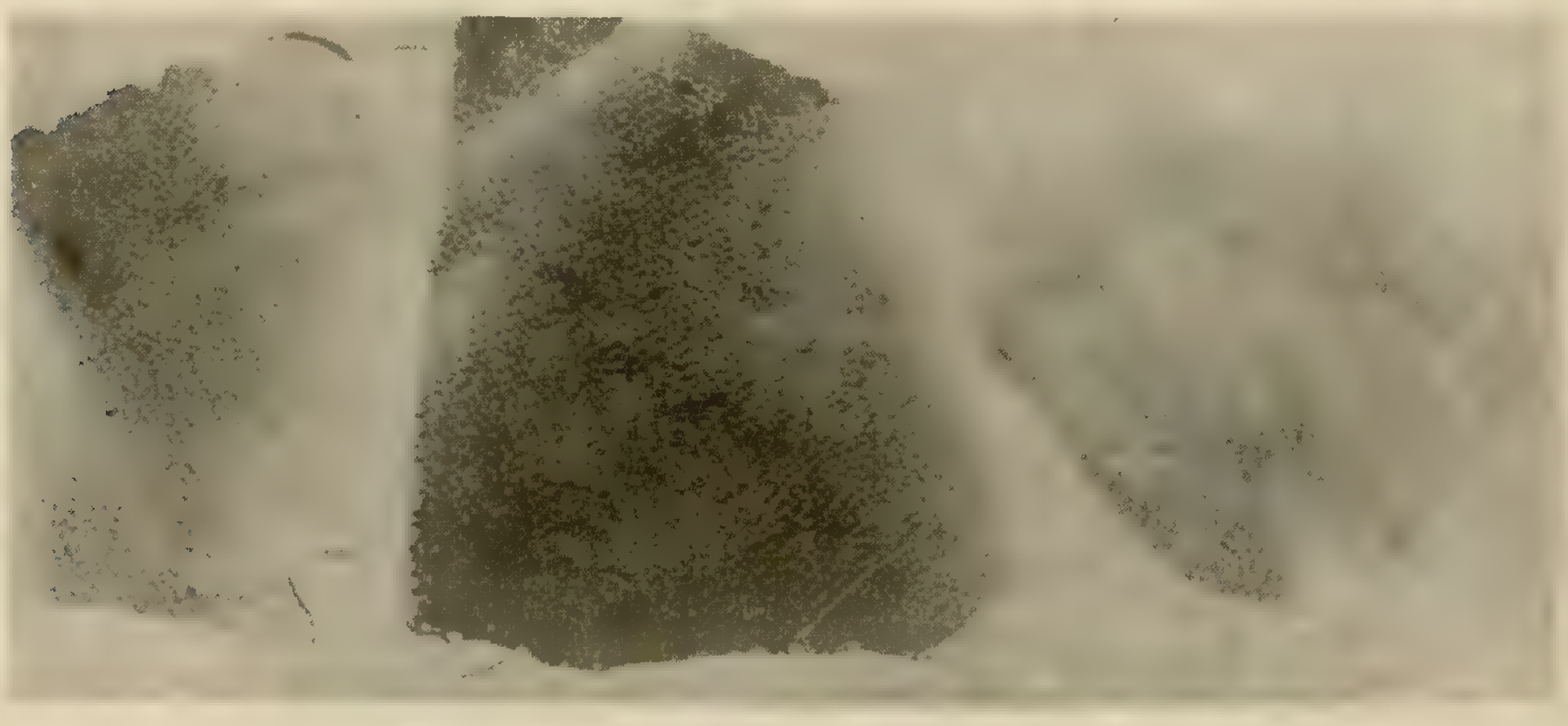


FIG. 15.—*Aloe ferox:* from same negative as fig. 14; ×2

smaller bundles at irregular intervals are probably responsible, but the principal cause is that the parenchyma cells formed at the close of a growing period are slightly smaller and have slightly thicker walls.

I wrote to Professor SCHÖNLAND, Director of the Albany Museum at Grahamstown, South Africa, and to Mr. E. E. GALPIN, formerly of Queenstown but now of Naboomspruit, Transvaal, South Africa, inquiring about climatic conditions in the localities from which the material was secured. Professor SCHÖNLAND, to whom I am also indebted for material of *Aloe ferox*, wrote as follows:

> There are two maxima of rainfall, in October and November, and in March and April; but this comes out only when the averages of a number of years are worked out and give a wrong picture of the relation of the flora to our rainfall. It is true that the winter, from the middle of June to the middle of September, is generally dry; but I have known good rains in these three months. Last year (1920) the October-November rains failed us; we had

good rains at Christmas, then drought to the middle of March, and then good soaking rains. The yearly amount of rainfall is often very interesting. In 1899 we had a serious drought up to Christmas, and then 12 inches in 24 hours. The long and short of it is that we live in what might be called the fag end of the summer rain area; but it can best be described as an area of uncertain rains.

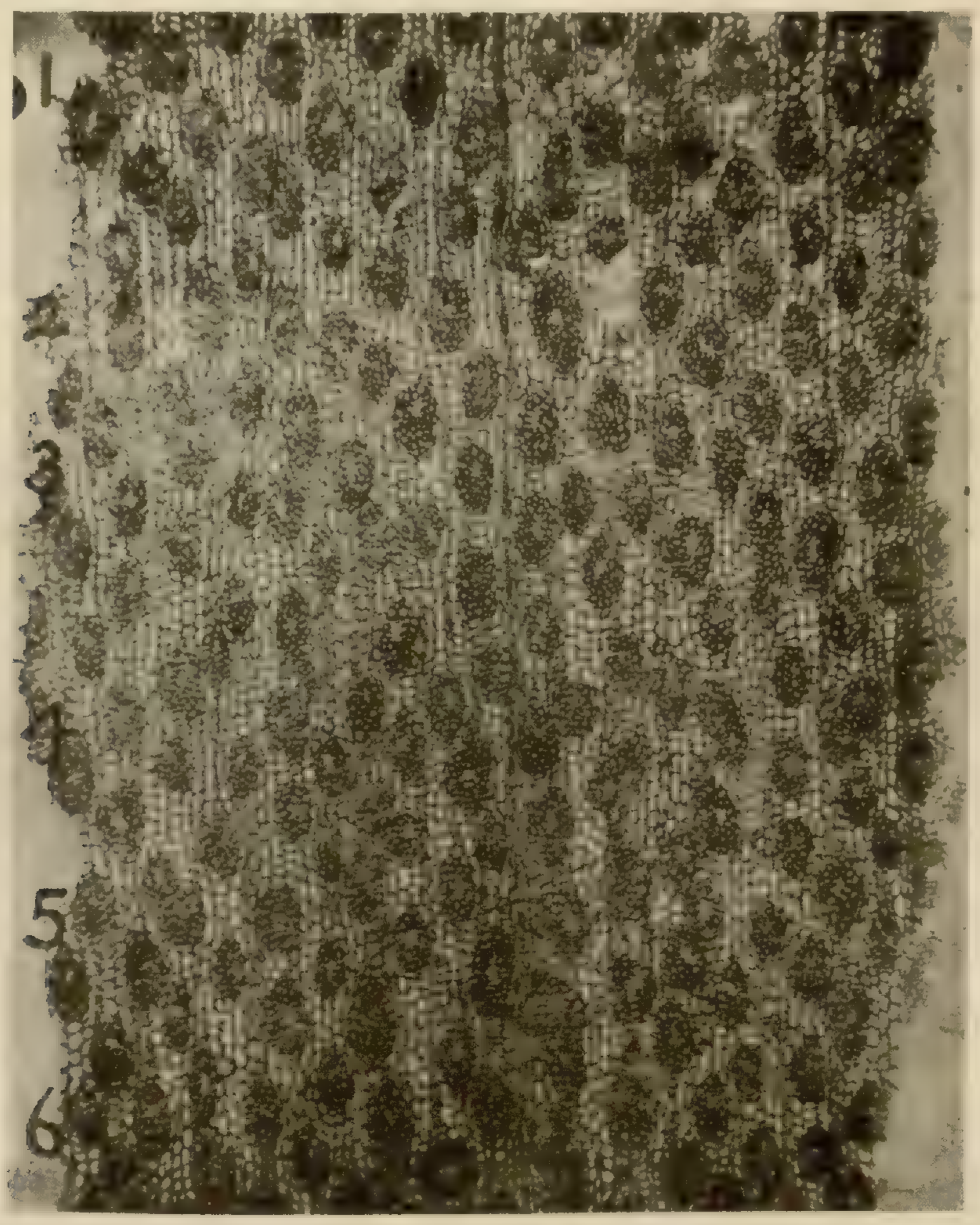


FIG. 16.—Small portion of region of secondary bundles, including six growth rings, numbered 1–6; ×10.

Mr. GALPIN replied as follows:

Around Grahamstown and the coastal districts generally, the seasons are very equitable, with no winter frosts and the rainfall fairly equally distributed throughout the year. Like the whole of South Africa, they have their lean years and their fat years, with deficient or generous rainfall, as the case may be. Added to this, at long but irregular intervals, perhaps a quarter of a century, more or less, for reasons as yet quite unexplained, there may come a period of prolonged drought or of exceptional rains. These remarks apply to Cathcart as well, but in that district, with an altitude of about 4000 ft., the seasonal changes are greater, with hot summers and frosty nights in winter.

The rainfall is also very much greater in summer than in winter, although the difference between dry and wet seasons is not so marked up here, nearer the tropics. They get a certain amount of rain during the winter months, while at Naboomspruit, with an annual rainfall of 25 inches, usually not a drop falls from the end of April to the beginning or even the end of October.

These two accounts, written by botanists who have made a prolonged study of the South African flora, show that the climatic conditions in the region where *Aloe ferox* grows are somewhat erratic. Large specimens were seen at Junction Farm in the Transkei, near Cathcart, and the negative from which fig. 1 was made was taken on the Windvogelberg, overlooking the town of Cathcart; but no material was collected. Judging from Mr. GALPIN'S account, specimens from the Windvogelberg would show more sharply marked rings than one would be likely to find in plants from the Grahamstown region where this material was collected. Both accounts, however, would lead one to expect the irregularities which appear in the rings of material collected near Grahamstown. Irregularities may be seen in figs. 14 and 15, and in fig. 16, where the position of the six rings is marked by the numerals 1–6.

Whether other species of *Aloe* would show rings or not could be determined very easily by one who is within reach of material. A few slides of *A. pleuridens* show a couple of faint rings. *Dracaena Hookeriana*, collected at East London, less than 100 miles south of Cathcart, shows secondary wood but no growth rings. It would be interesting to see the condition in *Aloe Bainesii*, the trunk of which may reach a diameter of a meter in less than thirty years. A specimen of *Yucca* with a zone of wood a centimeter in diameter, growing in the greenhouse, showed no growth rings; but such rings could hardly be expected in a greenhouse, where conditions are so uniform. One wonders whether the failure to find growth rings in woody monocotyls may not be due to the fact that they are mostly tropical and subtropical, out of the University zone, so that observations are likely to have been made upon greenhouse material. That there are growth rings in *Aloe ferox* is beyond question, and this is believed to be the first account of such rings in any monocotyl.

UNIVERSITY OF CHICAGO

INVASION OF VIRGIN SOIL IN THE TROPICS[1]

DUNCAN S. JOHNSON

(WITH TWO FIGURES)

This note is concerned with the revegetation of a tropical valley which was denuded of plants by a flood and later filled with detritus from a landslide. Acknowledgments are due to Messrs. H. A. GLEASON, WILLIAM HARRIS, M. A. HOWE, E. P. KILLIP, W. R. MAXON, and PERCY WILSON for the identification of plants collected in the Cascade Valley; to E. P. KILLIP and WILLIAM SEIFRIZ for taking photographs of the valley; and to JONAS WALKER, a Jamaican collector, for gathering plants growing in the valley in December.

The Blue Mountain region of Jamaica was subjected, in November 1909, to several days of nearly continuous torrential rains, such as apparently occur there only once or twice in a century. On November 8, 1909, there was a rainfall of 18.3 inches in 24 hours at the Cinchona Station, and this downpour continued into the next day, until 27 inches had fallen. The rainfall was undoubtedly heavier still on the higher peaks of the Blue Mountains which drain into the valley under discussion.

The floods arising from these tremendous rains caused striking changes in the topography, and in the plant covering of many considerable areas on both the north and the south sides of the Blue Mountains. In the first place, many small streams rose two or three meters above the normal level, and scoured their rocky banks clean of vegetation, aside from larger trees, for many meters on either side. In the second place, there were landslides from the wooded mountain sides, and especially from the cultivated coffee fields, which completely carried away soil and vegetation from scores of acres on the south side of the mountains. These landslides not only left great scars, showing the bare rock on the formerly tree-covered mountain sides and in the coffee fields

[1] Botanical contribution from the Johns Hopkins University, no. 70.

lower down, but they also filled in whole valley bottoms with the rock and gravel washed down from above. The amount of water and of débris carried with it was sufficient to wash away or bury out of sight most of a large and substantially constructed stone and concrete "coffee works" near the Cascade River.

The effect of the flood and landslides on the topography and vegetation of the valley of the Cascade River, a normally small mountain stream, located about three miles east of the Cinchona Botanical Station, was briefly described in a note published in 1910.[2] At that time, which was but six months after the flood, the floor of this valley was still a barren waste, covered with pebbles and broken rock fragments of all sizes, ranging from that of a pea up to bowlders a meter in diameter. The only plants evident at this time were a few widely scattered seedlings of *Bocconia frutescens* and still fewer seedlings of half a dozen other dicotyledons, such as grow on the hills beside the valley. The largest of these plants were only 2 or 3 dm. high. In other words, the valley bottom, which in 1903 and 1906 I had seen covered with a forest consisting of large trees together with dozens of types of shrubs and herbs, was in 1910 an all but absolute desert. The forest had been completely washed away or buried, and there was left a truly virgin soil, with no trace of humus, which bore but the barest sprinkling of young seedlings.

After studying the conditions in this and other valleys in 1910, and taking into account the abundant rainfall and frostless climate of the region, it was concluded that the floor of the Cascade Valley would probably be recovered with a dense vegetation, although perhaps not with a fully developed forest, in a score or two of years. It was realized, of course, that many of the forest plants, being dependent on an abundant humus, would not find satisfactory conditions there for many years, because of the slowness with which this type of soil is developed.

On a trip to Jamaica, in July 1919, I again visited the Cascade Valley, and expected to find that, during the nine years that had elapsed, the few plants that were starting on the newly deposited gravel in 1910 had multiplied greatly, and that many new species

[2] Jour. New York Bot. Gard. 11:273. 1910.

would be establishing themselves among those first invaders. Many of the possible invaders of the valley, found on the neighboring hills, have a long growing season. There are some species that grow actively from February to September, while still others grow practically throughout the whole year.[3] Because of this long growing season and the possibility of some humus washing down from the surrounding hills, it was assumed that by 1919 the soil of the valley floor would be well hidden by a plant covering.

FIG. 1.—Looking east across Cascade Valley, showing sparse vegetation, which sparseness would be still more evident if viewed from above.

My surprise was great, therefore, when I found hardly more than a tenth of the gravelly bottom of the Cascade Valley hidden by plant foliage. The soil between these leafy plants, it is true, was not absolutely bare. There were a few very small patches of lichens and mosses. There was also a chroococcaceous alga, *Gloeocapsa magma*, which formed smooth encrustations often several square decimeters in extent on the pebbles and bowlders. This alga is present not only near the streams but across the whole floor of the valley. When dry *Gloeocapsa* has a rather dirty or chocolate brown color, but when wet it becomes a glistening velvety layer of a dark maroon color. It evidently thrives on these bare

[3] SHREVE, F., Publication no. 199, Carnegie Inst. Wash. pp. 51–52. 1914.

rocks, although they may often be exposed to a scorching sun for many hours daily and be without rain for days or even several weeks together. There is a copious dew in the valley each clear night, however, while on cloudy nights the fog probably condenses on the rocks and plants of its floor. It is likely that *Gloeocapsa* may thus be able to carry on photosynthesis and growth for some hours each day, even without rain.

The bareness of the valley bottom recalled that of the more barren of the stony deserts of Arizona as they appear in early summer. The general aspect of this valley differed from that of these deserts in the absence of cacti and of all larger woody plants. No plants of this valley exceeded a meter or two in height except where, at the very edges of the valley, considerable top soil, that had washed down from the hillsides, afforded better conditions for plant growth. Here several species of shrubs grew to two or three meters in height, and in wetter soil considerable stands of *Arundo* sp. had established themselves (fig. 1, *x*). The shrubs and cane together made a conspicuous verdant border to the generally desert-like valley floor.

When the floor of the valley, especially the portion along the trail from the junction of the Cascade and Green rivers to Farm Hill Coffee Works, was more carefully examined, the scattered vegetation was found to include the following plants:

ALGAE

Gloeocapsa magma (Breb.) Kütz.

PTERIDOPHYTA

Dryopteris oligophylla Maxon
Blechnum occidentale L.
Gymnogramme tartarea (Sw.) Desv.
Trismeria trifoliata (L.) Diels
Pityrogramma calomelaeana (L.) Link
Pteris longifolia L.
Aneimia adiantifolia (L.) Sw.

DICOTYLEDONEAE

Piper sp.? (shrub or tree)
Pilea microphylla L. (Liebm.) (annual to perennial)
Iresine celosioides L. (half shrubby)
Begonia acuminata Dryand. (half shrubby)
Asclepias curassavica L. (perennial herb)
Asclepias nivea L. (perennial herb)
Philibertella clausa (Jacq.) Vail (shrubby vine)
Duranta plumieri Jacq.(shrub 2-3 meters)
Verbena bonariensis L. (perennial herb)
Solanum torvum Sw. (half sh rubby)
Maurandia scandens A. Gr. (shrubby vine)

MONOCOTYLEDONEAE
Arundo (saccharoides Gr. ?)

Ageratum conoyzoides L. (annual)
Ageratum houstonianum Mill. (annual)
Vernonia acuminata Less. (half shrubby)
Vernonia permollis Gleason (half shrubby)
Mikania scandens L. (Wild.) (shrubby vine)
Eupatorium triste DC. (half shrubby)
Baccharis scoparia Sw. (shrubby)
Pluchea odorata L. (Cass.) (half shrubby)
Bidens incisa Ker. (annual)
Senecio discolor (Sw.) DC. (shrubby)

There were thus seven species of ferns, of which *Dryopteris oligophylla*, *Blechnum occidentale*, and *Aneimia adiantifolia* were rare, less than a score of each being seen where we crossed the valley. *Pityrogramma calomelaena* and *Gymnogramme tartarica* were more frequent; while *Trismeria trifoliata* was represented by dozens of specimens in the moister soil, and of *Pteris longifolia* there were still more numerous clumps in the drier spots along the trail across the valley. From the size of many of the fern plants seen it seems clear that they have been established for some time. In the cases of *Gymnogramme* and *Trismeria*, where fronds a meter high were seen, it was hard to believe that such plants could have arisen from a prothallus in nine years. Yet they must have done so unless it is assumed that old rhizomes have persisted in the soil to push up through the gravel, or that pieces of rhizomes have been washed down by the flood of 1909 or subsequent lesser ones. The first supposition seems negatived by the fact that no ferns were seen in 1910, six months after the flood, and also by the fact that each clump of a fern consists of but one or a few branches and leaf clusters. This latter feature tends to confirm the impression gained from the character of the soil, namely, that these ferns have started *in situ* from prothallia.

All the seed plants found in the valley, except *Arundo* along the stream at the foot of the cliff, were dicotyledons. By far the most important of these was the composite *Vernonia permollis*. Scores of clumps of this, from quite young plants up to those 2 m. high, were found scattered across the valley. They grew beside the larger rocks and often also formed rather definite rows along

the small dry gullies, which during the rainy season drain the raised middle of the valley floor that lies between the main stream on the west and the branch stream that comes in from the east. This ironweed is the most prominent plant of the valley, not only because of its abundance but also from its size. It is this plant, for example, that forms the major component of the clumps shown in fig. 1. The three more prominent plants after *Vernonia permollis* are *Bocconia frutescens* (already grown to 2 or 3 m. in height), *Solanum torvum Sw.* (often 2 m. high), and *Vernonia acuminata*

FIG. 2.—Looking north over upper Cascade Valley, showing scars left on south side of Blue Mountains by landslides.

(about 2 m). These larger plants are sometimes mingled with the *Vernonia permollis*, although much fewer than the latter, but may also be scattered sparingly by themselves over the valley floor.

Of the less prominent seed plants of the valley some fifteen species were found. These, with their relative abundance, are: *Piper* sp.? (two or three young plants), *Pilea microphylla* L. (Liebm.) (rather frequent), *Iresine celosioides* L. (sparse), *Begonia acuminata* (very few), *Asclepias curassavica* L. and *A. nivea* L. (both infrequent), *Philibertella clausa* (Jacq.) Vail. (a dozen plants seen), *Duranta plumieri* Jacq. (half a dozen plants), *Verbena bonariensis* L. (few), *Solanum torvum* Sw., *Maurandia scandens* A. Gr. (occa-

sional at edges of valley), *Ageratum conyzoides* L. and *A. houstonianum* Mill. (rare), *Mikania scandens* L. (Wild.) (infrequent), *Eupatorium triste* DC. (few), *Baccharis scoparia* sp. (a dozen or so), *Pluchea adorata* L. (Cass.) (not infrequent), and *Bidens incisa* Ker. (frequent). All of these plants, with the possible exceptions of the *Pilea* and *Bidens*, were far less abundant than any of the four species mentioned in the preceding paragraph. Most of these fifteen plants are also smaller species, which likewise makes them less conspicuous in the vegetation of the valley. The *Duranta*, *Solanum*, and *Baccharis* are now as large as the species of *Vernonia*, but not as numerous. The climbing forms *Philibertella*, *Maurandia*, and *Mikania* of course are rather long, having already reached and spread over the tops of the largest plants near them. Many individuals of these fifteen species, as for example those growing in unusually dry situations, were dwarfed, and thus showed by their stunted form that they were not finding optimum conditions in the sterile soil and dry exposed situations afforded by the gravelly floor of the valley.

It is to be noted that, contrary to the accepted rule for invaders of new soil areas, as stated by WARMING,[4] the plants now established in the Cascade Valley are not mostly annuals or biennials. Instead they are chiefly perennials, and in fact shrubby or half-shrubby ones. Although this is true, it is to be noted also that not one arborescent form has yet been found, unless some of the young plants of *Piper* seen should prove to belong to one of the more tree-like species of this usually shrublike genus.

In this area of virgin soil there are present right through the year all of the climatic factors, such as moisture, heat, and light, that are needed for the production of a rich vegetation. This is evident from the dense forest that has developed in the adjoining valleys and even on the hills immediately overhanging the Cascade Valley itself. It was for these reasons that the writer was rather surprised, on revisiting this valley in 1919, at the slowness with which it is being recovered with vegetation. He was surprised not only at the relatively small number of new individuals, but especially at the very small number of species that had established themselves in the

[4] Oecology of plants. p. 356. 1909.

decade. It was anticipated in 1910 that certain plants which require abundant humus would not be able to settle at once on its bowlders and gravel, nor could epiphytes soon find the necessary trees to perch in. That the many mosses, ferns, and seed plants that grow all about the valley, not only in similar gravelly and stony soil along the trails, but even in the crevices of every rugged cliff and crag of the neighboring hills, should prove incapable of promptly and completely colonizing this valley was quite unexpected.

The decisive causes responsible for this slowness of revegetation have not been determined. It may be remarked in the first place that browsing by animals is a negligible factor in the development of the vegetation, since such animals are not allowed to run free in this region. Furthermore, it does not seem probable that the chemical nature of the rock can be the prime cause of this phenomenon. It is conceivable that at a later stage the soil formed by disintegration of the rock, which is an epidosite (or epidote gneiss), may determine the types of micro-organisms living in the soil and so the kinds of humus produced. The fact that a rather varied series of some thirty species of plants have been able to establish themselves in this valley shows that the soil, which is probably of fairly uniform chemical character throughout, is not especially unfavorable to plants. The distribution of the plants now growing in the valley seems rather to be related to the physical character of the soil. Plants are found growing where finer soil particles have accumulated. Probably the most important hindrance to the increase of the vegetation is instability of the soil, which, in most areas of this rather steeply sloping valley, is being constantly changed, by erosion at some points and by deposit at others.

It seems clear that in the future development of the plant covering of this valley the existing vegetation after a time will establish more fixed conditions in areas now occupied. This will give the mycorhizal fungi and soil bacteria, which cannot thrive in this sterile gravel, a sufficient amount of vegetable matter on which to feed. There will then probably be a decided acceleration both in the spread of the plant species now present, and in the introduction of new species. The writer hopes, during the coming decade, to be able to study further and to report on the progress of the revegetation of this valley.

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PECTIC MATERIAL IN ROOT HAIRS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 286

CAROLINE G. HOWE

It has been observed for some time that many soils show some acidity, that plants are able to take up much more mineral matter than can easily be extracted from soil, and also that although fertilizers are made up of soluble salts, the virgin soils are composed largely of difficultly soluble salts, and after they have been cultivated a few years, yield as full crops if not fuller than those treated with ordinary fertilizers. This has given rise to the question whether there may not be something in the structure of the root hair which enables it to change the difficultly soluble salts to such a form that they can be dissolved and taken into the plant; and since root hairs are so ephemeral, any chemical effect they may have upon the soil will not be present very long in one place.

Since many soils were found to be somewhat acid, and yet most plants cannot grow in an acid medium, two explanations were offered for this phenomenon; first, that there was an acid in the soil which, for lack of a better name, was called humus acid; and secondly, that negatively charged colloidal particles either in the plant tissues or in the soil broke up the salts and released the acids into the soil.

BAUMANN and GULLY (cited by SKENE 6) investigated this matter with peat and mosses, and found that when put into a sodium chloride solution, these plants were able to absorb the positive ion and thus release the chlorine, which, combining with the hydrogen ion, made hydrochloric acid. SKENE (6) made similar tests upon sphagnum, using copper chloride solution, and found that the moss had taken up the copper, releasing the chlorine. which again formed hydrochloric acid. WIELER (8) also tested the higher plants, such as the needles of the pine, the leaves of the horse chestnut, American oak, and yellow lupine, and found that they were all acid, and concluded that the decaying vegetation

would explain the presence of acid in the soil. Again, ODÉN (3) found in the plants that he examined a gelatinous material of the nature of pectic acid, and MANGIN (1) found that pectose is often formed in young cells before cellulose, and that the middle lamella is calcium pectate. He (2) also found that pectose can be changed to pectic acid or pectin by gently heating in 2 per cent hydrochloric acid.

SAMPSON (5), in investigating abscission of the leaf of *Coleus*, found that there was calcium pectate in the middle lamella just before the time of abscission, but that the calcium was lacking at abscission, and discovered that this was due to the pectic acid forming so much more rapidly than the calcium was supplied that the middle lamella was broken down. Miss ROBERTS (4) also examined root hairs of a number of seedlings grown in moist air and found that they all had a layer of pectic material outside the cellulose wall, and often at the tip of the hair there was a layer of callose.

In order to determine whether this condition is general, the root hairs of twenty economic plants grown in sand and in loam were examined, and those of a few seedlings grown in Knop's solution. These seedlings were selected with the idea, first, of getting as great range as possible, and secondly, of comparing several in the same or closely related genera. These root hairs were tested microchemically for cellulose with iodine and 70 per cent sulphuric acid, which turns cellulose bright blue; for callose with resorcin blue, which causes callose to swell and to turn blue; for acidity with neutral red (and later with the Clark and Lubs indicators to determine the degree of acidity); for calcium pectate with ammonium oxalate, which unites with the calcium pectate when calcium oxalate crystals and ammonium pectate are formed; and for pectic material in general with ruthenian red. Of the special forms of pectic material chiefly found in plants, pectose is found especially in young tissues, is insoluble in water, but can be changed to pectic acid or pectin by gently heating for twenty minutes in a 2 per cent solution of hydrochloric acid. Pectin is soluble in water, and pectic acid is soluble in a 2 per cent solution of potassium hydroxide when gently heated for twenty minutes.

TABLE I

Seeds	Ammonium oxalate and calcium pectate form calcium oxalate crystals and ammonium pectate		Ruthenian red for pectic material, in general; 2 per cent potassium hydroxide for pectic acid, which is dissolved by it; 2 per cent hydrochloric acid for change of pectose; pectin soluble in water	
	Test for calcium oxalate crystals		Test for pectic material	
	Loam	Sand	Loam	Sand
Beans (Kentucky wonder)	Many crystals	Many crystals	Thick layer; pectose changed mostly to pectic acid	Pectose changed to pectin chiefly, some to pectic acid
Beans (Bush lima)	Few crystals	Few crystals	Pectose changed chiefly to pectin, some to pectic acid; thin layer	Thin layer; changed chiefly to pectic acid
Beans (Pole lima)	Many crystals, especially in older roothairs	Many crystals	Thick; pectose changed to pectic acid	Thick; pectose changed to pectic acid
Beans (Golden wax)	Very few crystals	Very few crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Cabbage (Chinese)	Crystals fairly abundant	Many crystals	Pectose changed largely to pectin	Same as in loam
Cabbage (Early Jersey Wakefield)	Very few	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Carrot (Danver's half long)	?	?	Thick layer; pectose changed to pectic acid	Same as in loam
Corn (Yellow bantam)	Many crystals	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Cress (Doubled curled)	Very few	Very few, even less than in loam	Pectose changed to pectic acid	Pectose changed to pectin chiefly, some to pectic acid
Cucumber (Early fortune)	Almost no crystals	Almost no crystals	Thin layer; pectose changed mostly to pectin, some to pectic acid	Thin layer; pectose changed mostly to pectin

TABLE I—*Continued*

Seeds	Ammonium oxalate and calcium pectate form calcium oxalate crystals and ammonium pectate		Ruthenian red for pectic material, in general; 2 per cent potassium hydroxide for pectic acid, which is dissolved by it; 2 per cent hydrochloric acid for change of pectose; pectin soluble in water	
	Test for calcium oxalate crystals		Test for pectic material	
	Loam	Sand	Loam	Sand
Egg plant (Black beauty).....	Many crystals	Many crystals	Thick layer; pectose changed to pectic acid	Same as in loam
Lettuce (Mignonette).....	Few crystals	Many crystals, especially near tip	Pectose changed chiefly to pectic acid	Same as in loam
Parsnip (Hollow crown).....	Very few crystals	Very few crystals	Pectose changed chiefly to pectic acid	Same as in loam
Peas (Telephone)..	Many crystals	Many crystals	Pectose changed to pectic acid	Same as in loam
Radish (Sparkler)..	Many crystals	Many crystals	Pectose changed to pectic acid	Same as in loam
Squash (Golden Hubbard).....	Many crystals	Many crystals	Pectose changed chiefly to pectic acid, some to pectin	Same as in loam
Squash (Giant summer crook neck).....	Number of crystals	Number of crystals	Pectose changed to pectic acid	Little pectic acid: pectose changed to pectic acid
Swiss Chard (Lucullus).....	Few crystals	Few crystals	Pectose changed to pectic acid	Some pectic acid; pectose changed to pectic acid
Tomatoes (Ponderosa).....	Few crystals	Few crystals	Pectose changed to pectin	Thin layer; pectose changed to pectic acid
Watermelon (Cole's early)..	Many crystals	Many crystals	Some pectic acid; pectose changed to pectic acid	Same as in loam

In general, the root system was found more extensive on those seedlings grown in sand, and the root hairs were much longer.

It was also more difficult to find the young root hairs on the roots grown in sand. Pectic material was found in the outer layer of all the root hairs; some of it was in the form of calcium pectate in practically all the root hairs, much was in the form of pectose, and it was difficult to determine with certainty whether some was in the form of pectic acid. By the application of 2 per cent hydrochloric acid the pectose was changed to pectic acid except in a few instances when some was changed to pectin, and the calcium pectate was broken down to calcium chloride, allowing pectic acid to be set free. Why pectose is changed sometimes to one form and sometimes to the other is still an unsolved problem.

Callose forming an inner lamella of the wall was found in all the root hairs, being somewhat thicker at the tip, especially of the younger root hairs. The hairs grown in the two media did not differ essentially in these respects, except that the callose was somewhat thicker at the tips in loam than in sand. No cellulose was found in the root hair walls. As the root epidermal cell bulges to form a hair, the cellulose inner lamella apparently stretches to its capacity, then breaks, and no more cellulose is formed. It may be that under other conditions more cellulose would be formed.

The root hairs gave an acid reaction in all cases both in the loam and in the sand, but usually somewhat higher in the loam than in the sand. According to the P_H value, they ranged between 6.8–6.0 in the sand and in the loam, and in some cases in loam between 6.0–5.2.

The seedlings of only four species were grown in Knop's solution, and the hairs were quite numerous and symmetrical. Before the seeds were placed for germination in the Knop's solution, it was tested and found to have an acidity of 6.8–6.0. After the seedlings had grown, both the root hairs and the solution were tested for acidity. The root hairs showed about the same degree of acidity or a little less than that of the root hairs grown in the soil, while the solution was also less acid than the original, even becoming alkaline in three of the cases. These root hairs had the same structure as those grown in loam and sand, except that the callose was thicker at the tips and in two of the cases the pectose was changed to pectin.

TABLE II*

Seeds	Resorcin blue changed callose to blue		Acidity, P_H value, by use of Clark and Lubs' indicators	
	Callose		Acidity	
	Loam	Sand	Loam	Sand
Beans (Kentucky wonder)	Thick layer, especially on young hairs	Thick layer, especially on young hairs	6.0–5.2	6.0–5.2
Beans (Bush lima)	Thick layer	Thick layer	6.0–5.2	6.0–5.2
Beans (Pole lima)	Thick layer	Thick layer	6.0–5.2	6.0–5.2
Beans (Golden wax)	Fairly thick layer, especially on young hairs and at tip	Thick layer	6.0–5.2	6.8–6.0
Cabbage (Chinese)	Thin layer	Thin layer	6.8–6.0	6.8–6.0
Cabbage (Early Jersey Wakefield)	Thin layer; found on young root especially	Same as in loam	Nearer 6.0	Nearer 6.8
Carrots (Danvers)	At tip only, in young hairs, then all around	Thick in older hairs	6.0–5.2	6.0–5.2
Corn (Yellow bantam)	Thick layer, especially at tip	Same as in loam	4.6–4.4	6.0–5.2
Cress (Doubled curled)	Thick layer	Thin layer	6.0–5.2	7.6–6.8
Cucumber (Early fortune)	Thick layer	Thick layer	6.0–5.2	6.8–6.0
Egg plant (Black beauty)	Thick layer, especially at tip	Thick layer	6.0–5.2	6.0–5.2
Lettuce (Mignonette)	On younger hairs especially	Same as in loam	6.0–5.2	6.0–5.2
Parsnip (Hollow crown)	Thick layer, especially at tip	Same as in loam	6.8–6.0	7.6–6.8
Peas (Telephone)	Thick layer	Thick layer	?	6.0–5.2
Radish (Sparkler)	Some, but root hairs rather old	Layer at tip	6.8–6.0	7.6–6.8
Squash (Golden Hubbard)	Thick layer, thicker at tip	Thin layer, thicker at tip	6.0–5.2	6.8–6.0
Squash (Giant summer crookneck)	Thin layer	Fairly thick layer	6.0–5.2	6.8–6.0
Swiss Chard (Lucullus)	Thin layer, thicker at tip	Same as in loam	6.0–5.2	6.8–6.0
Tomatoes (Ponderosa)	Thick layer, especially on young hairs	Same as in loam	6.0–5.2	6.0–5.2
Watermelon (Cole's early)	Thick layer, especially on young hairs	Same as in loam	6.0–5.2	6.8–6.0

* Cellulose was not found in any of the root hairs.

From these experiments it would seem that some of the acidity of the soil is due to pectic material in the root hairs, and that this

may help change some of the difficultly soluble salts, such as tricalcium phosphate, to a soluble form that can be used by the plant.

TABLE III*

SEEDLINGS GROWN IN KNOP'S SOLUTION

SEEDS	AMMONIUM OXALATE AND CALCIUM PECTATE FORM CALCIUM OXALATE CRYSTALS AND AMMONIUM PECTATE	RUTHENIAN RED FOR PECTIC MATERIAL, IN GENERAL; 2 PER CENT HYDROCHLORIC ACID FOR CHANGE OF PECTOSE; 2 PER CENT POTASSIUM HYDROXIDE FOR PECTIC ACID	RESORCIN BLUE FOR CALLOSE
	Test for calcium oxalate crystals	Test for pectic material	Test for callose
Cabbage (Early Jersey Wakefield)	Many crystals	Thick layer all around; pectose changed mostly to pectin, some to pectic acid	Thick layer; thicker at tip
Cucumber (Early fortune)	Few crystals	Thick layer; pectose changed to pectic acid at tip, to pectin at sides	Very thick layer all round
Radish (Sparkler)	Many crystals	Pectose changed to pectic acid at tip, to pectin at sides	Thick layer, especially at tip
Muskmelon (Rockyford)	Few crystals	Pectose changed largely to pectin	Fairly thick layer

* Cellulose was not found in any of the root hairs.

TABLE IV

SEEDLINGS GROWN IN KNOP'S SOLUTION

SEEDS	ACIDITY, PH VALUE BY USE OF CLARK AND LUBS' INDICATORS	
	Root hairs	Solution
Cabbage	6.8–6.0	7.6–7.2
Cucumber	7.2–6.8	8.4–7.6
Radish	6.8–6.0	8.4–7.6
Muskmelon	6.8–6.0	6.8–6.0

Summary

1. No cellulose was found in the root hairs of the species studied.

2. The root hairs grown in both loam and sand have a layer of pectic material on the outside, and within a layer of callose, thicker in some plants than in others, and usually a little thicker at the tips.

3. The pectic material in most of the cases at first is in the form of calcium pectate or pectose; pectic acid could not be detected with certainty. The pectic layer is somewhat thicker in loam than in sand.[1]

4. The root hairs are somewhat acid in the forms studied, and there is a tendency to be slightly more acid in loam than in sand.

5. Whether the acidity of the root hair can be ascribed to the presence of pectic material or to some other cause has not been yet determined with certainty.

Acknowledgement is due to Dr. SOPHIA H. ECKERSON for her suggestions and criticism during the progress of this study.

EAST ORANGE, N.J.

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[1] The pectose is usually changed to pectic acid by the hydrochloric acid.

DESTRUCTION OF MOSSES BY LICHENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 287

FRANK P. McWHORTER

(WITH PLATE XIII)

The deep-rooted conception of lichens as typical examples of symbiosis has induced workers along ecological lines to overlook the occurrence in xerarch successions of early stages which are dominated by the parasitism of lichens on mosses. This preliminary paper is intended to describe certain cases of lichen parasitism, and to emphasize the accuracy of FINK'S definition of lichens: "A lichen is a fungus which lives during all or part of its life in parasitic relation with an algal host, and also sustains a relation with an organic or inorganic substratum."

The writer's attention was first called to this situation when trying to separate some *Cladonia* lichen material from a moss colony in which it was growing. The intimacy of the mixture suggested that the lichen might be to some extent parasitic on the moss. Such phenomena seem to have been noticed previously by BONNIER,[1] who shows that spores of lichens are known to germinate on moss protonemas and eventually to attack and kill them. He suggests the occurrence of such parasitism in nature on a large scale.

Moss-lichen colonies were chosen for study, in which both elements were intimately mixed, illustrating cases of dominance on the part of one or the other. It was often impossible to determine the exact species or even genus of the mosses concerned, because of the poor condition of the vegetative body and lack of reproductive organs. Mosses hampered by invading lichens seldom produce spores. Representative lichen-moss mixtures consisting of species

[1] BONNIER, GASTON. Germination des spores des Lichens sur les protonemas des Mousses et sur des Algues differents des gonidies du Lichen. Compt. Rend. Soc. Biol. Paris. 40:541–543. 1888.

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of *Dicranum*, *Bryum*, *Grimmia*, or *Fissidens* with *Cladonia*, *Physcia*, or *Amphiloma* have been collected. The following species determinations of mosses based on vegetative characters may be taken as probable: *Dicranum scoparium*, *Bryum caespiticium*, *B. argenteum*, *Grimmia apocarpa*, *G. pennsylvanica*, and *Fissidens adiantoides*. Especially important among the lichens concerned are *Cladonia cristatella*, *C. baccillaris*, *C. pyxidata*, *Physcia stellaris*, *P. obscura*, and *Amphiloma lanuginosum*.

All previous observations along this line are based on cultures and the examination of teased materials. The method employed consisted of imbedding and sectioning moss-lichen colonies, the resultant serial sections giving a veritable moving picture of the conditions in the colonies. The striking destruction of moss tissues is evident from sections 10 μ or more in thickness, but to judge the extent and nature of the haustorial action it is necessary to have sections 3 μ or less in thickness. Many kinds of fixatives were used to show to the best advantage the various tissues concerned; no one fixative gave the most satisfactory fixation for all. The fungus elements of the lichen fix well in chromoacetic; the algal elements in hot bichloride of mercury. The location of the nucleus in the algal cells, and the condition of plastids in the moss show well in aceto-formalin. The cell wall structures of all the tissues showed best in aceto-formalin. Very weak Flemming's solution gave excellent results in the young tissues of the moss. The three tissues, moss, fungus, and alga, can be sharply differentiated by a carefully balanced Flemming's triple stain. For wall studies nothing proved better than a contrasting safranin-analin-blue stain. With this stain the cell wall changes and the haustorial action may be clearly demonstrated. Slides so stained were easily photographed by suitable combinations of yellow and green filters. In addition to the section studies, a long series of cultures was run with *Amphiloma* and other lichen genera to see how readily and under what conditions they would attack a moss host.

The destructive action of lichens on moss may be seen from figs. 1 and 2. These were from 10 μ sections of intimate mixtures of *Cladonia* lichens with *Dicranum*, *Grimmia*, and other mosses,

in which the moss appears plastered over by the lichens. The apical development of the moss has been stopped. The lichen hyphae could be traced through the old moss tissue where they forced their way intercellularly.

A very constant feature of the lichen growth on mosses is the clinging of the lichen hyphae to the thickened walls of the moss. This seems to be of great significance in the eventual destruction of the moss colony. It is not the meristematic tissue that seems particularly desirable to the lichen fungus, but the thickenings of the moss walls. The case seems homologous with the destruction of wood by a polyporous fungus, where the lignified part of the wood is especially attacked, and the cellulose walls are left almost untouched. In the mosses the young walls are pure cellulose; the thickenings are of pectin. Thin sections of all the moss-lichen colonies studied showed the hyphae imbedded in the pectin. There is no evidence that the hyphae have been covered over by the forming pectin layers, but it seems obvious that the hyphae have taken their position by dissolving out the pectin. The figures of *Amphiloma* on *Grimmia* show a case of this, but *Amphiloma* is more destructive than most lichens, and in places has completely destroyed the moss. When sharply stained in safranin and analine blue, the pectinized part of the walls stains a strong red, so that penetration of the bluish stained hyphae may be plainly followed. In some colonies, even when the lichens appear to be literally plastered over the mosses, the lichen hyphae were found to be confined to the pectinized regions, and the cellulose walls to be intact; then the lichens are exerting a smothering effect carried on through a saprophytic rather than a parasitic action.

Lichen fungi sometimes become truly parasitic on their moss hosts. This is especially true of *Amphiloma*, which is shown in fig. 6. Here the lichen is an intracellular parasite. *Amphiloma* haustoria soon break down the plastids, even in old moss tissues. *Amphiloma* seldom attacks the meristematic tissues. Under some conditions *Physcia obscura* may send hyphae of non-rhizoidal nature into the meristematic moss tissues. *Physcia* also may so incorporate moss into its thallus, that the epidermis of the lichen

develops on the lower side of the moss leaf and the rest of the lichen on the other side, the moss becoming a veritable layer of the lichen. In such cases the moss leaf is eventually destroyed.

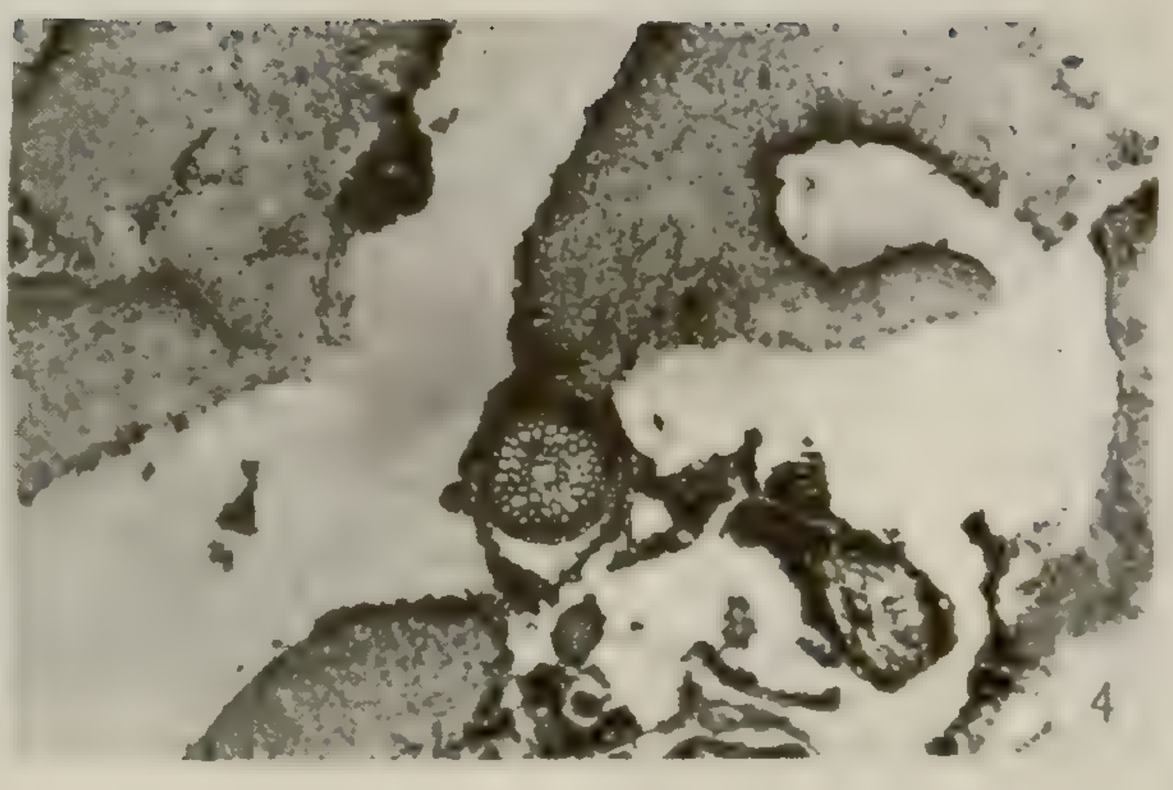
The great opportunity for the parasitizing of mosses by lichens, as they grow together in nature, cannot be over emphasized. For the most part the lichens develop on the leaves of the moss. Moss colonies in which apparently no lichens are present, when sectioned or teased out almost invariably show tiny young lichens developing in their leaves. Hundreds of lichens have been seen developing from soredial masses, but very few from spores, hence it is concluded that moss inhabiting lichens depend on soredia rather than on spores for reproduction. BONNIER'S observations on the ability of lichens to germinate on, and eventually to kill moss protonema, have already been mentioned. Since the protonemal stage is a transient one, it probably does not take place in nature to any great extent. The germination of lichens on moss leaves is the rule, so far as cases where lichens eventually plaster themselves over the mosses are concerned. The young lichen hyphae become attached from their first formation. The environmental factors control the future appearance of the colonies. From cultures and field observations it is concluded that water is the dominating factor of the control. Almost any moss colony, apparently free from lichens, when grown in semimoist conditions, but occasionally allowed to dry out, in a few weeks will produce young lichens visible to the naked eye.

If these observations are borne in mind, it is easy to see why so often the ideal lichen-moss-fern sequence is not carried out, since the sequence is broken up by lichen stages in which the lichens are more or less parasitic on the moss. If the rock surface is rough enough, visible life may be initiated by moss, and a lichen stage come in secondly. In any event a well established moss stage may be crowded out by a more or less parasitic lichen mass, which gives a secondary lichen stage succeeding the moss.

Summary

Lichens are able to destroy moss colonies. The destruction is partly due to true parasitism and partly to smothering.

1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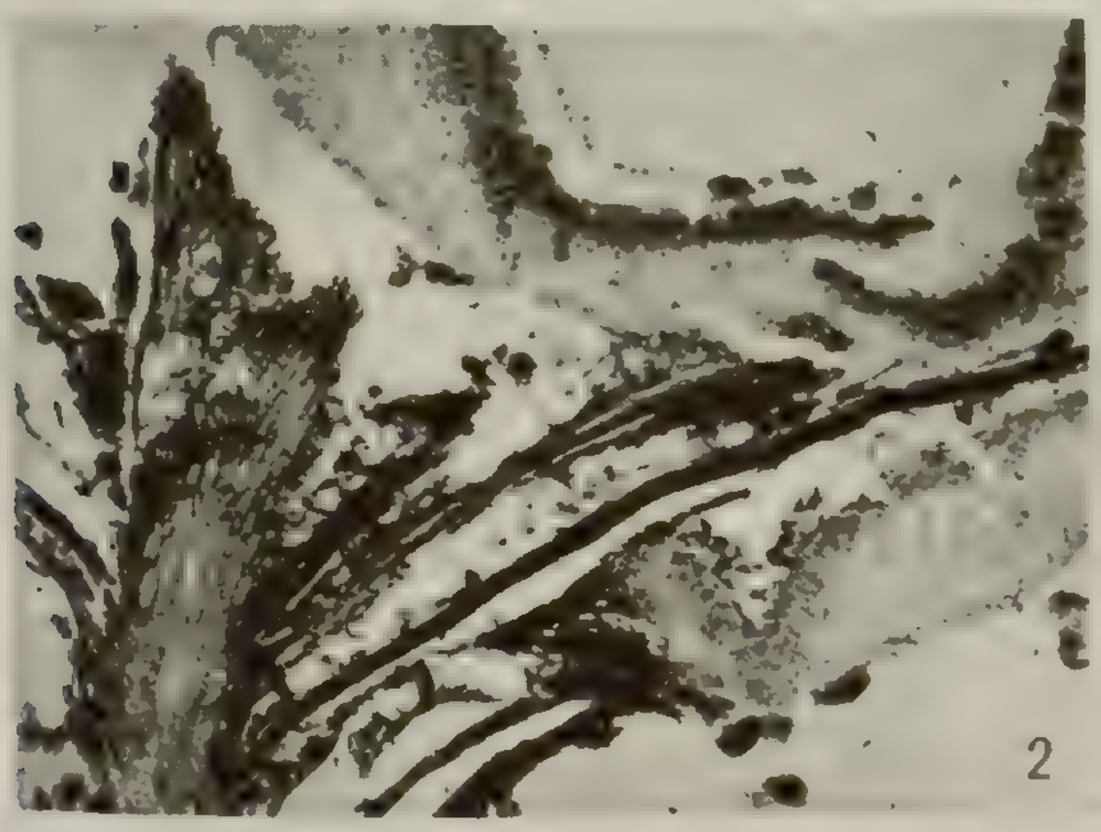
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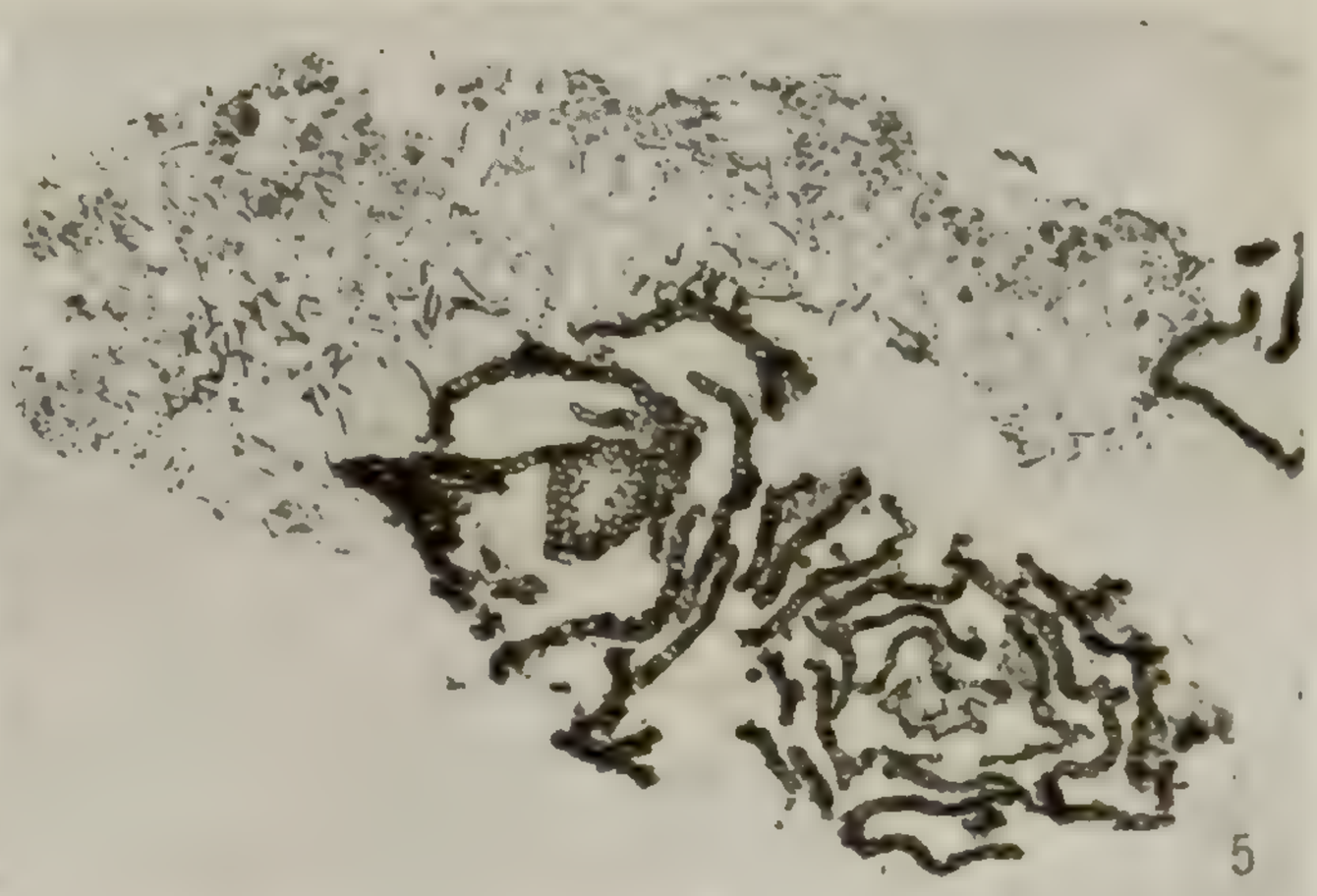
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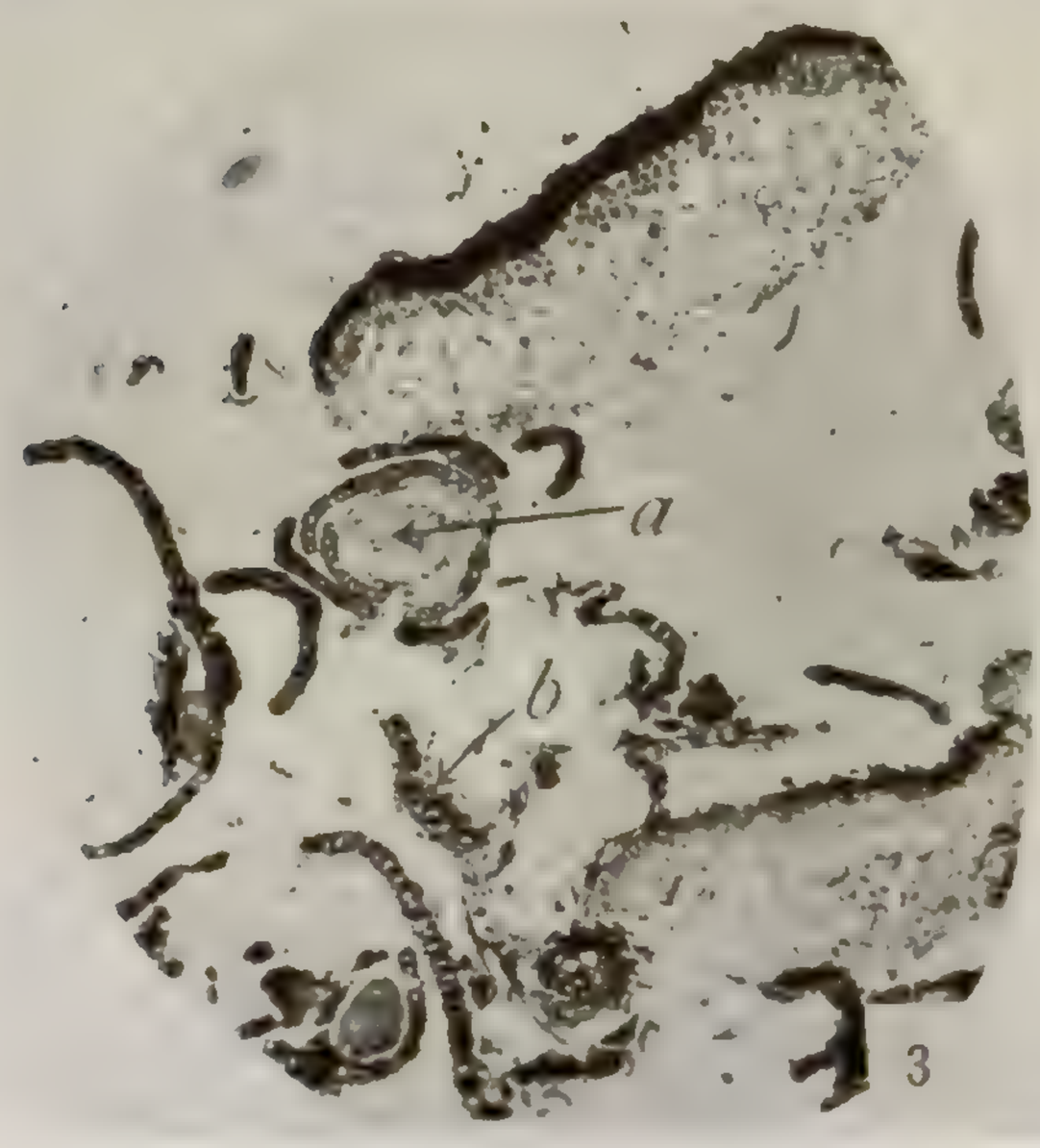

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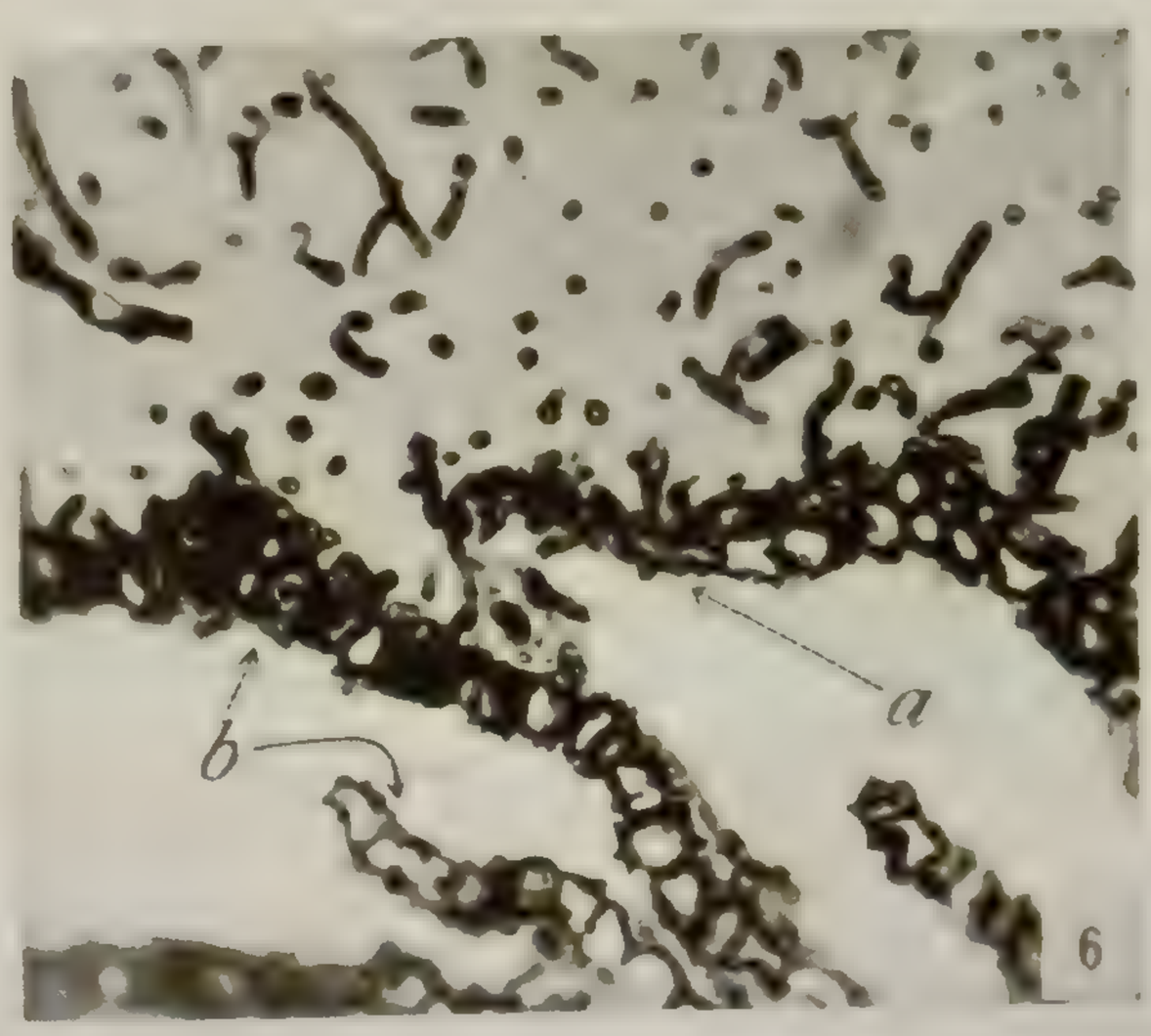

6

The development of lichens in moss colonies makes possible the coming in of a lichen stage after the moss associations.

Great obligation is due to Professor W. J. G. LAND for suggestions in regard to the technique used, and Dr. GEO. D. FULLER for aid in the preparation of the manuscript and for reading the proof.

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EXPLANATION OF PLATE XIII

The illustrations are all photomicrographs selected from a much larger number showing similar conditions.

FIG. 1.—*Cladonia pyxidata* on moss, probably *Dicranum;* vertical section through colony showing moss plant with leaves cut to pieces and apical growth stopped by action of lichen.

FIG. 2.—*Cladonia pyxidata* on an unrecognizable moss; organization of moss leaves destroyed by action of lichen; lichen hyphae penetrated intercellularly through moss tissues.

FIG. 3.—Section of a moss-lichen mixture cut parallel to surface of colony, showing lichen (*Cladonia*) hyphae penetrated into cells of apical region of moss (*Grimmia*); hyphae indicated by arrow *a* could be traced through serial sections to lichen mass just above; *b*, moss leaf strongly attacked by hyphae.

FIG. 4.—Section from lower part of colony cut parallel to surface, showing lichen hyphae tending to fuse with pectinized walls of moss tissues.

FIG. 5.—Section through a *Amphiloma-Grimmia* mixture; *Amphiloma* has organized on moss leaf; this lichen probably destroys more moss than any other.

FIG. 6.—Portion of fig. 5 more highly magnified, showing: *a*, how lichen may completely destroy moss cells; *b*, how hyphae dissolve pectinized layer of cell walls of moss.

ANNUAL RINGS OF GROWTH IN CARBONIFEROUS WOOD

WINIFRED GOLDRING

(WITH PLATE XIV)

In a discussion of anatomical structure and climatic evolution, JEFFREY[1] emphasizes the absence of annual rings in Cordaitean wood from the Carboniferous in latitudes south of England as indicative of uniformity of climate, in contrast with the conditions in the Triassic period, in which coniferous wood with annual rings is found as far south as Arizona. His statement is as follows:

> In the Paleozoic trunks which are supplied by the geological formations of Southern Canada the organization of the wood shows great uniformity, and there are no modifications of structure which indicate any periodicity in annual conditions of growth. The truth of this statement is well illustrated by . . . . wood of a Cordaitean form from the Permo-Carboniferous of Hampton, Prince Edward Island. The presence of clear zones of periodic growth is, however, frequently found in regions of higher latitude . . . . . The next illustration shows the organization of a Carboniferous Cordaitean wood (*Mesoxylon*) from the northern part of England, and consequently of considerably higher latitude (54° N. in contrast to the 46° N., the latitute of Prince Edward Island). The annual rings in the wood from the English Carboniferous are clearly marked.
>
> For comparison with the situation revealed by the Cordaitean wood from Northern England, a trunk from the Triassic of the southwest region of the United States (Arizona) is shown . . . . . The annual rings are not so distinct in the photomicrograph as they appear on the weathered end of the actual petrified specimen. It will be clear from the information supplied in this case that as far south as Arizona in the Triassic annual rings were more or less clearly marked. A noteworthy variation in the annual temperature in that somewhat remote epoch is thus indicated.[2] This situation presents an interesting contrast to the climatic conditions which prevailed in the region of Prince Edward Island toward the end of the Paleozoic. If the situation be summarized, it is clear that in the later Paleozoic the difference between 46° N. and 54° N. means the presence in the higher latitude of annual rings and their absence in the lower one. On the other hand, in the beginning of the Mesozoic (the Triassic), even at a distance of 10° south of the latitude of Prince Edward Island, annual rings were quite clearly developed.

[1] JEFFREY, E. C., The anatomy of woody plants. 1917.

[2] This might indicate variation in moisture instead of temperature.

Recently BERRY has come into possession of part of a trunk of *Cordaites* from Bartlesville, Oklahoma, which shows annual rings of growth quite distinctly. The specimen comes from the Upper Pennsylvanian (below the Americus formation). The location is described as follows in a letter from the donor, Mr. GILBERT HART:

> As near as I can judge, the trees are confined to a rather limited belt, and are rather common there. . . . . The trees are found always below the Americus. As yet I have seen none surely in place; the nearest to the original position was in talus just below the first heavy limestone in the Admire formation. I feel sure that this is almost the true horizon.

The latitude of Bartlesville, Oklahoma, is 36°45′ N., about 10° south of the Prince Edward Island locality, practically the latitude of the Triassic forest of Arizona, where is found the coniferous wood showing more or less clearly marked annual rings. If the occurrence in Arizona argues for a "noteworthy variation in the annual temperature" in this area during the Triassic, then the annual rings in the trees of the Oklahoma forest would indicate the same for the end of the Carboniferous.

So far as known, the Oklahoma forest is the most southern occurrence of Carboniferous wood with annual rings of growth which has been noted; but such occurrences have previously been noted in wood from the Carboniferous, or earlier, in latitudes as far (or farther) south as Prince Edward Island. PENHALLOW,[3] in his discussion of North American species of *Dadoxylon*, states that, of the eighteen species now entitled to recognition, three show more or less clearly defined growth rings, while in the remaining fifteen they are obscure or obsolete. Of the species discussed in this paper one, *Cordaites pennsylvanicum* (Dawson) Penhallow, showing distinct growth rings, comes from the Carboniferous at Pittsville, Pennsylvania (41°30′ N.). Two other species, *C. Hamiltonense* Penhallow and *C. Clarkii* Dawson from the Devonian (Genesee shales) of Ontario County, New York (43°N.), show obscure growth rings. In the second species, however, they are, sometimes wanting. Both the Pennsylvanian and New York localities are much farther south than the English (54° N.) or Prince Edward Island (46° N.) areas.

[3] Trans. Roy. Soc. Can. **6**:57. 1900.

KNOWLTON,[4] in a survey of all the described species of *Cordaites* and *Dadoxylon*, describes twenty-four species as showing growth rings either distinctly or indistinctly. Of these, *Cordaites ouangondianum* Dawson from the Middle Devonian of New Brunswick and *Dadoxylon* (*Cordaites*) *annulatum* Dawson of the Middle Carboniferous of Nova Scotia must be excluded because the original descriptions were based on a complete misinterpretation of structural features (PENHALLOW, p. 56). Of the other species, nine are from the Carboniferous, the remainder from the Permian. Of the Carboniferous species, seven are from latitudes south of England, and of these four species are from latitudes as far, or practically as far south as Prince Edward Island, as follows: Nova Scotia (46° N.), three species; Niederburbach in Upper Alsace (47°45′ N.), one species showing distinct rings of growth. Most of the Permian species range in latitudes from 50°15′ N. to 51° N., but one species, with distinct growth rings, is recorded from Val d' Ajol, Department of Upper Sâone, France (47°40′ N.).

These data show that the extreme southern extension of a variable annual temperature in the Triassic period is not particularly remarkable. As far back as the Middle Devonian (Genesee) there must have been noticeable variations in climate in fairly low latitudes, in order to effect even the slight variations in wood formation noted, while in the Carboniferous the development of distinctly marked annual rings of growth indicates a pronounced seasonal variation in the climate of that period, even in far southern latitudes. This is also shown, but less markedly, in the Permian.

The specimen from the Upper Carboniferous of Bartlesville, Oklahoma, represents part of a trunk of a tree of considerable size, for in the section of trunk preserved, a radius of 5.5 inches of wood is shown with neither pith on one side nor cortex on the other. PENHALLOW gave the name *Cordaites recentium* to an undescribed species from the Permian or Permo-Carboniferous of Prince Edward Island, which Sir WILLIAM DAWSON had regarded as related, if not identical with *C. materiarium* Dawson from the Upper Carboniferous of Nova Scotia, Newfoundland, Illinois, etc. The species was not figured, but after a comparison of PENHALLOW's description

[4] Proc. U.S. Nat. Museum. **12**:601–617. 1890.

with thin sections of the Oklahoma trunk, there seems no real justification for a separation of the latter from the Prince Edward Island species, in spite of the great distance between the two localities. The original description follows:

Cordaites recentium (Dawson) Penhallow

Transverse.—Tracheids 47×53 μ broad, the walls much reduced by decay.

Radial.—Ray cells all of one kind, about equal to two tracheids; the lateral walls with round pits about one (?) per tracheid; the cells conspicuously narrower at the ends.

Bordered pits in a single row, compact, large, compressed and transversely oval or oblong, 15.6×22 μ, the orifice very variable, from oblong to round, often eccentric, but typically round and central. When distant the pits are round and smaller.

Tangential.—Rays medium, 1–2 seriate, the very broad cells 41 μ, thin-walled, round and squarish.

PENHALLOW makes no mention of the occurrence of annual rings of growth, which are very distinctly shown in the Oklahoma specimen. The rings of growth shown in the transverse section are variable in width; one has a width of 3 mm., a second 8 mm., and 6 mm. of a third are shown. The growth rings show very well on the weathered surface of the trunk; in one place the growth rings have the following successive widths: 3 mm., 3.5 mm., 7.5 mm., 3.5 mm., 3 mm., 3.5 mm., 3 mm., etc.; in another place, 4 mm., 4 mm., 4 mm., etc. On the whole, therefore, the growth rings are of about even width.

The bordered pits in a single row on the radial walls of the tracheids distinguish *C. recentium* from *C. materiarium*, in which the pits are numerous throughout the tracheids, chiefly in two. sometimes in three or four rows. The ray cells are narrowed at the ends, but not conspicuously so, and are equal to 2–6 tracheids in the Oklahoma specimen, as in *C. materiarium*, the longer cells being more frequent. The pits on the lateral walls are round, and so far as can be ascertained, one to a tracheid as described by PENHALLOW. The rays are numerous and in general very long, composed of from two to at least forty-seven cells superimposed upon each other and tapering toward each end. The rays are described as "1–2 seriate," but usually they are uniseriate. In no

place have they been found biseriate throughout. The biseriality is usually confined to the middle of the ray, although it may also occur at one or both ends; often it is confined only to the depth of one to three cells.

C. recentium resembles *Dadoxylon antiquum* Dawson (Upper Carboniferous of Nova Scotia) in the possession of bordered pits in one row, but differs from it, among other things, in the possession of practically uniseriate rays, whereas *D. antiquum* has multiseriate rays two to four cells wide. *D. prosseri* Penhallow (Permian, Chase County, Kansas) has numerous uniseriate rays (biseriate in part), but the bordered pits are smaller, and although they may occur in one row on the tracheid walls, are chiefly in two rows.

Photographic reproductions of transverse, radial, and tangential sections of the wood of this species are shown in the accompanying plate.

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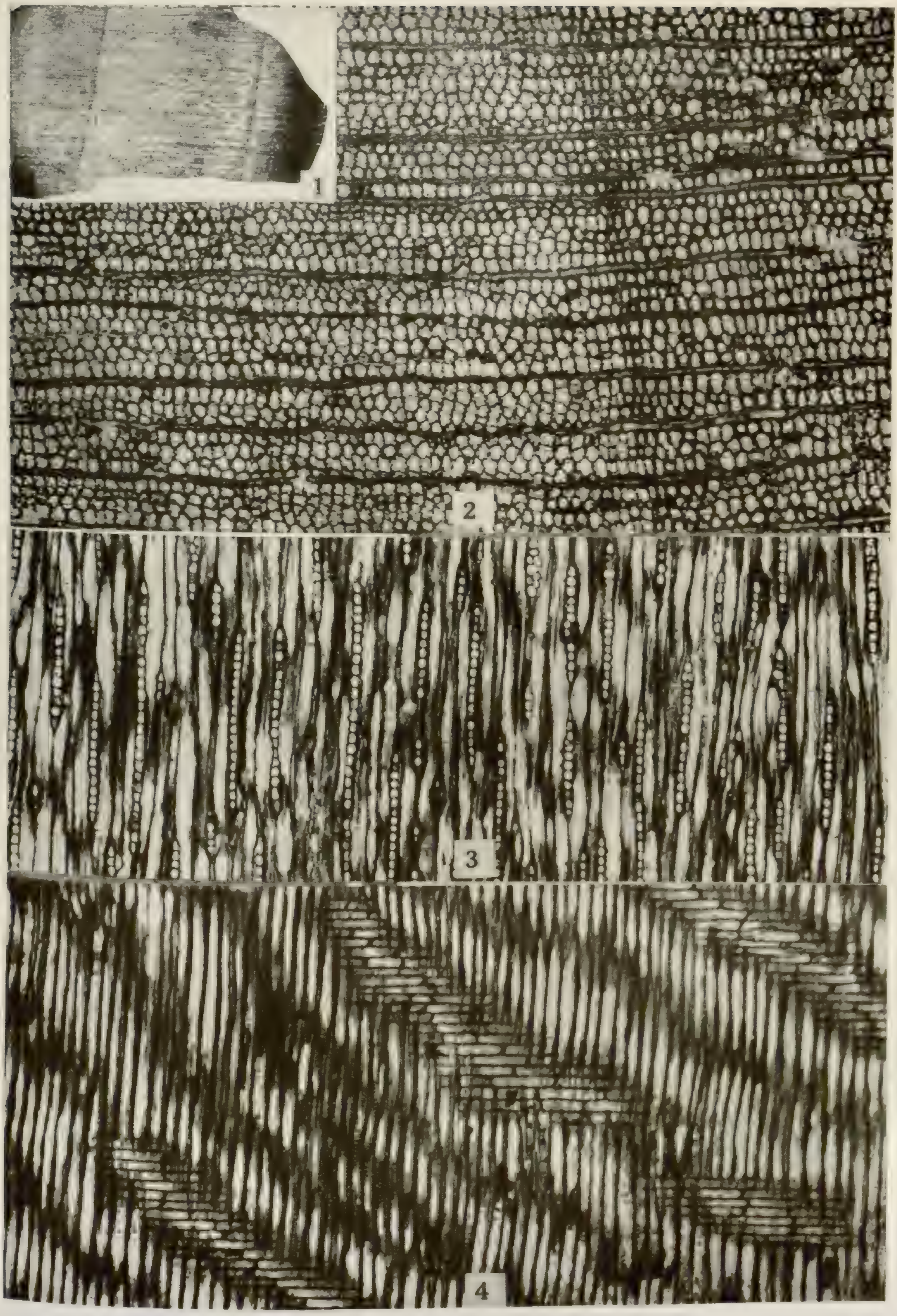
EXPLANATION OF PLATE XIV

FIG. 1.—Transverse section, ×3.
FIG. 2.—Transverse section, ×50.
FIG. 3.—Tangential section, ×50.
FIG. 4.—Radial section, ×50.



GOLDRING on CARBONIFEROUS WOOD

CURRENT LITERATURE

BOOK REVIEWS

Cytology

A book on cytology from the botanical standpoint has long been needed, and consequently botanists will welcome the vigorous, suggestive presentation of the subject by SHARP.[1] The zoological side is also presented, so that everywhere the differences and similarities in plant and animal cells are kept before the reader.

This is the first time that such a comprehensive treatment of the whole subject of cytology has been attempted by a botanist. The chapter headings, which indicate the scope of the work, are as follows: Historical sketch; Preliminary description of the cell; Protoplasm; The nucleus; The centrosome and blepharoplast; Plastids and chondriosomes; Metaplasm, polarity; Somatic mitosis and chromosome individuality; The achromatic figure, cytokinesis, and the cell wall; Other modes of nuclear division; The reduction of chromosomes; Fertilization; Apogamy, apospory, and parthenogenesis; The rôle of the cell organs in heredity; Mendelism and mutation; Sex; Linkage; Weissmanism and other theories. Any discussion or comment on details would require such an undue amount of space, that reference will only be made to the book itself.

The illustrations are numerous and exceptionally well drawn. Accompanying the descriptions of vegetative mitosis, reduction of chromosomes, and the rôle of the nucleus in heredity are numerous diagrams which will be appreciated both by students and investigators. The large number of new figures and diagrams is refreshing, and, as one turns the pages, he sees at a glance that the book owes little to shears and paste. Each subject is followed by a very full bibliography arranged alphabetically. The citations are unusually complete. The index is also commendable, with reference to figures in full faced type and with words which might be unfamiliar to some botanists or zoologists followed by an explanatory word in parenthesis, as *Eloesis* (palm), *Ectocarpus* (brown alga), *Enchenopa* (bug), etc.

SHARP'S own contributions in the field of cytology, his skill as a practical technician and artist, as well as his experience in teaching the subject have fitted him for the production of this book, which will be indispensable to botanists, and should be of great value to those zoologists who feel the need of an authoritative presentation of the botanical side of cytology.—C. J. CHAMBERLAIN.

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[1] SHARP, L. W., An introduction to cytology. 8vo. pp. xiii+452. New York: McGraw Hill Book Co. 1921. $4.

In Lower Florida wilds

A volume by SIMPSON,[2] described as a naturalist's observations on the geology, geography, and life of the more tropical part of the state, presents much scientific information in an attractive popular manner, well illustrated with plates from good photographs. Two chapters should be of particular interest to plant ecologists, since the one outlines the plant succession from the pine lands to the "hammocks," while the other gives a picture of the primeval forest of semitropical type. The author's opinion that the broad level evergreen forest of the "hammock" is the true climax vegetation seems well founded, while his emphasis upon the destructive and retrogressive effect of fire appears to furnish a part, at least, of the explanation why such vegetation has not dominated a larger portion of the peninsula.

The tale of the evolution of the land is attractively told, and seems as scientifically accurate as the story of the succession in the forests.—GEO. D. FULLER.

NOTES FOR STUDENTS

Organic acids and anthocyanin formation.—COMBES,[3] ROSE,[4] NICOLAS,[5] and other workers have found that anthocyanin formation is accompanied by increased oxygen fixation. MIRANDE,[6] KEEBLE[7] and ARMSTRONG, and MIEGE[8] have found that anthocyanin formation occurs regularly in tissues containing comparatively large quantities of oxidases. Later work, such as that of COMBES,[9] WILLSTÄTTER,[10] and EVEREST,[11] has shown that anthocyanins

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[2] SIMPSON, C. T., In Lower Florida wilds. 8vo. pp. xv+404. *pls. 64. maps. 2.* New York: Putnam's Sons. 1920. $3.50.

[3] COMBES, R., Les echanges gazeux des feuilles pendant la formation et la destruction des pigments anthocyaniques. Rev. Gen. Botanique **27**:177–212. 1910.

[4] ROSE, E., Étude des echanges gazeux et de la variation des sucres et des glucosides au cours de la formation des pigments anthocyaniques dans les fleurs de *Cobaea scandens*. Rev. Gen. Botanique **26**:257–270. 1914.

[5] NICOLAS, G., Contribution a l'étude des relations qui existent, dans les feuilles, entre la respiration et la presenxe de l'anthocyane. Rev. Gen. Botanique **31**:161–178. 1919.

[6] MIRANDE, M., Sur l'origine de l'anthocyanine, deduite de l'observation de quelques insectes parasites des feuilles. Compt. Rend. Acad. Sci. **145**:1300. 1907.

[7] KEEBLE, F., and ARMSTRONG, E. F., The rôle of oxidases in the formation of anthocyan pigments of plants. Jour. Genetics **2**:277–311. 1912.

[8] MIEGE, E., Recherches sur les principales espéces de *Fagopyrum*. Thesis for Doctorate. Paris. 1914.

[9] COMBES, R., Production experimentale d'une anthocyane identique a celle qui se forme dans les feuilles rouges en automne, en partant d'un compose extrait des feuilles vertes. Compt. Rend. Acad. Sci. **147**:1002–1004. 1913.

[10] WILLSTÄTTER, R., Über die Farbstoff der Bluten und Fruchte. Sitz. Ber. Akad. Wiss. 402–411. 1914.

[11] EVEREST, E., The production of anthocyanins and anthocyanidins. Proc. Roy. Soc. B. **87**:444–453. 1914.

can be produced from flavones by reduction. In the light of this work, Miss KOHLER[12] was led to believe, as NICOLAS and others previously had been, that anthocyanin formation should be correlated with organic acid accumulation, her contention being that organic substances such as carbohydrates were oxidized to organic acids, thereby reducing certain flavones and causing anthocyanin formation.

The evidence for the accumulation of organic acids during formation of red plant pigment has been more or less contradictory. WIESNER[13] and KRAUS[14] have found that acidity of the cell sap increases during autumnal reddening of leaves. ASTRUC[15] has shown that acidity descreases in petals of flowers during the reddening process. The tissues immediately beneath the red epidermis of apples were found to be less acid than tissues beneath a green epidermis in the same fruit, as determined by RIVIERE and BAILHACHE.[16] BERTHELOT and ANDRE[17] state that the amount of free acid in the plant as determined by titration of expressed juice bears no relation to the total amount of organic acid in the plant, as for the most part the acids are combined as salts of plant bases. Miss KOHLER also objects to titration of expressed juice because of the tendency of the alkali used to combine with phenolic compounds such as tannins and anthocyanins. After several unsatisfactory attempts to precipitate the phenolic compounds by the use of hide powder, zinc acetate, and analgesine (antipyrine), Miss KOHLER found that free organic acids could be quantitatively dialyzed out of the expressed juice and therefore used this method in her work. The acids were then titrated with a base and calculated as free organic acid. Combined organic acids were determined, using oven dried samples of tissue, by heating at dull red heat in a muffle. A known quantity of N/10 sulphuric acid was then added to the ash to neutralize the bases liberated by the combustion, and the acid residue titrated with alkali to find how much acid was neutralized by the ash. The figure obtained in this way was added to that of the free organic acid and the sum placed under the caption "total organic acids." Total organic acids determined in this way were found to increase in corollas of *Cobaea scandens* during the process of development from bud to mature flower, along with anthocyanin development,

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[12] KOHLER, DENISE, Étude de la variation des acides organiques an cours de la pigmentations anthocyanique. Memoir to Faculty of Science, Univ. Paris. 1921.

[13] WIESNER, J., Untersuchungen über die Herbstliche Entlaugung der Holzgewachse. Sitz. Ber. Akad. Wiss. **64**:465–510. 1871.

[14] KRAUS, C., Studien über die Herbstfarbung der Blätter und über Bildungweise der Pflanzensauren. Buchner's Repert. Pharm. **22**:273. 1873.

[15] ASTRUC, A., Recherches sur l'acidity végétale. Ann. Sci. Nat. Bot. **17**:65–109. 1903.

[16] RIVIERE, G., and BAILHACHE, G., De l'influence de la lumiere directe sur la composition chimique des fruits. Jour. Soc. Nat. Hort. France, IV. **9**:627. 1908.

[17] BERTHELOT, M., and ANDRE, G., Remarques sur la formation des acides chez les vegetaux. Compt. Rend. Acad. Sci. **32**:502. 1901.

when the flowers were allowed to remain attached to the plant. When detached there was no increase during the opening of the corolla. Leaves of *Ampelopsis tricuspidata* gathered September 17, October 1, and again on November 2, showed a progressive increase in total organic acids during the autumnal reddening. When allowed to redden detached from the plant, there was no accumulation of acids. Small plants of buckwheat, when germinated in the dark, showed a steady increase in total acids until the eighteenth day after germination. When exposed to light during this time, a red pigment developed in the hypocotyl axis, but no corresponding increase in total acids was found, either in plants attached to or detached from the parent stock. Miss KOHLER states that this fact may mean that the destruction of organic acids formed in this case is greater than their production.

It is to be regretted that Miss KOHLER has not included some similar determinations upon leaves which remain green under certain conditions and which redden under certain other conditions, in order that a comparison might be made. There is some doubt in the reviewer's mind that titration of ash, after incineration of plant tissue, gives an approximate value of the combined organic acids in the tissue before incineration. Plant tissue is a complex material. Salt combinations other than those of organic acids with inorganic bases may be altered greatly by incineration, and may leave a basic ash. Organic acids may as well be combined with organic bases within the plant and both would be lost on heating. There is even the possibility of a mixture of inorganic salts becoming more basic upon heating in a muffle. There is a tendency toward accumulation of mineral salts as the leaf ages during autumn, according to PALLADIN.[18] This accumulation might account for an increase in basicity of ash independent of color formation. In the same way migration of mineral salts into corollas and subsequent use of certain anions such as sulphates, nitrates, and phosphates in building complex compounds connected with reproduction may leave basic elements which combine in various ways and which would increase basicity of ash upon incineration. On account of the many criticisms which might be justly directed against this method of determination of combined organic acid, and on account of the insufficiency of our knowledge of complex plant compounds, it is hoped that the author of the paper will continue her studies, including some corollas which do not redden at the time of opening, and some leaves which do not redden in autumn, together with other methods for quantitatively determining the acids in question.—J. M. ARTHUR.

Vegetation of Lower California.—As the result of an expedition conducted by members of the United States Bureau of Biological Survey in 1905 and 1906,

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[18] PALLADIN, V. I., Plant physiology. p. 83. 6th. ed. transl. by LIVINGSTON, B. E., 1917.

NELSON[19] has given us a rather extensive account of the geography and resources of one of the least known regions of the continent. The larger portion of the report is occupied with an account of the exploration of the peninsula from north to south. Although 800 miles in length and from 30 to 100 miles in width, it possesses a population of little over 30,000, more than half of which is found in the extreme north on the delta plain of the Colorado River. Much of this sparcity of population is due to the essentially desert character of the peninsula as a whole. Rainfall records are very scanty, but show that there is rarely over 10 inches of annual precipitation, while over large areas from one to five years may pass with practically no rainfall. Some idea of the general aridity may be formed from the fact that throughout three-fourths of the entire length of the peninsula there are no forests whatever, only the tops of the higher mountains at the northern and southern ends being covered with trees. The only extensive forests are those contained in the northern area, where trees extend along a narrow belt 150 miles long on the higher slopes of the Juarez and San Pedro Martis Mountains, and within this area the merchantable timber does not cover more than 400 square miles. Here the more important species are *Pinus Jeffreyi*, *P. contorta*, *P. Lambertiana*, *Abies concolor*, and *Librocedrus decurrens*. Associated with them are other trees and shrubs, the same or similar species to those of southwestern California, often constituting a scattered chaparral.

The essentially desert character of the remainder of the peninsula is also shown by the inclusion of three-fourths of the entire land area within the Arid Lower Sonoran Life Zone and of more than half of the remainder within the Arid Tropical Zone. This zonal division also corresponds closely to the three main elements of the flora derived respectively from (1) the mountains and foothills of southern California, seen in the northern forests and scrub; (2) the deserts of the northwestern Sonora and southwestern United States; and (3) the lowlands and mountains of the southern Sonora on the mainland of Mexico.

Cacti appear to reach a climax in the Lower Sonoran, both in size and abundance. In his annotated list of species GOLDMAN[20] gives over 30 species belonging to 11 genera, varying in size from the smallest to such large forms as *Lemaireocereus eruca*, with huge, prostrate, caterpillar-like stems 6 or 8 ft. long, and the largest of the giants, *Pachycereus Pringlei*, more than 50 ft. high and three ft. in diameter. Many of the associated plants are the same as those of California, including species of the *Yucca*, *Agave*, *Fouqueria*, *Cercidium*, *Parkinsonia*, *Prosopis*, *Covillea*, and palms of the genera *Washingtonia* and

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[19] NELSON, E. W., Lower California and its natural resources. Mem. Nat. Acad. Sci. **16**:1–194. *pls. 1–31*. 1921.

[20] GOLDMAN, E. A., Plant records of an expedition to Lower California. Contrib. U.S. Nat. Herb. **16**:309–371. *pls. 103–133*. map. 1916.

Erythea, these last at the base of the mountains. In the southern third of the peninsula many distinctly tropical genera appear, such as *Ficus*, *Mimosa*, *Cassia*, *Albizzia*, *Jatropha*, *Haematoxylon*, *Lantana*, *Manihot*, and *Chiococca*.

Among the more remarkable endemic desert forms, two trees may be mentioned. One, belonging to the Anacardiaceae, *Pachycormus discolor*, is found in the extremely arid central section of the peninsula. Seldom 10 ft. in height, the branches often shoot out twice that distance from the trunk, while their thickness (1 ft. or more), their abrupt ending in a few short twigs covered with red flowers, "reminding one of the proboscis of an elephant holding a nosegay," give a remarkably grotesque appearance to the tree. The leaves are minute and fall off before the flowers are fully developed. The associated monotypic genus *Idria columnaris* is in the Fouqueriaceae, and appears as a tree reaching 50 ft. in height in a scattered open forest. In contrast with the preceding it has a straight columnar trunk, usually without large branches. Illustrations of these and many other interesting and unusual plants add much to the interest of both reports.—GEO. D. FULLER.

Rubus in New England.—BRAINERD and PEITERSEN,[21] recognizing that "*Rubus* is one of the most polymorphic genera in the entire plant kingdom," have presented the blackberry group of that genus as displayed in New England. The authors say that the remarkable variation in the number of species recognized in the various taxonomic works is due to too great reliance upon herbarium specimens, to failure to appreciate the variations due to environmental conditions, and to lack of appreciation of the extent of interbreeding. The present study is based upon data from material in the field, behavior in garden cultures and controlled plots, characters of the progeny of supposed natural hybrids, and behavior of progeny when artificially crossed. The result is that the authors recognize twelve valid species of New England blackberries, and a long list of hybrids:

In following up the experimental work, PEITERSEN[22] has reached the following conclusions: variations due to external factors are very marked; primordia of the prickle, glandular hair, and simple hair are present in all species: a large percentage of infertility occurs in most species, largely due to defective pollen; cross pollination is the rule in all species, all the species studied being either nearly or completely self-sterile; all the species are capable of inter-crossing under favorable conditions; duplicates of natural hybrids were produced artificially; the progeny of a number of so-called species segregated as hybrids.

The paper is a good illustration of the test of genetics applied to taxonomy.—J. M. C.

[21] BRAINERD, EZRA, and PEITERSEN, A. K., Blackberries of New England; their classification. Bull. 217, Vermont Agric. Exper. Sta. pp. 84. *pls. 36*. 1920.

[22] PEITERSEN, A. K., Blackberries of New England; genetic status of the plants. Bull. 218. Vermont Agric. Exper. Sta. pp. 34. *pls. 19*. 1921.

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OPTIMUM TEMPERATURES FOR FLOWER SEED GERMINATION[1]

GEO. T. HARRINGTON

(WITH TEN FIGURES)

The proper conditions for the germination of flower seeds is a subject upon which but little work has been published. During the spring of 1912, preliminary work was done in the seed laboratory of the United States Department of Agriculture on the temperature conditions best suited for the germination of a few of the more common flower seeds. During the winter and spring of 1913–1914, further work was done with the same species investigated in 1912, and with a few additional species. The publication of the results has been unavoidably delayed for several years. In the meantime, the recommendations included herein have been followed by the seed laboratory with good results.

The seeds included in the investigation were those of *Impatiens balsamina*, *Eschscholtzia californica*, *Iberis amara*, *Cosmos bipinnatus*, *Kochia scoparia*, *Delphinium ajacis*, *Calendula officinalis*, *Reseda odorata*, *Tropaeolum majus* and *T. minus*, *Viola tricolor*, *Petunia hybrida*, *Dianthus chinensis*, *Papaver* spp., *Portulaca splendens*, *Antirrhinum majus*, *Lathyrus odoratus*, and *Zinnia elegans*. Only a small number of samples of some kinds was included in the actual investigation. The subsequent experience of the seed laboratory, however, includes the germination of such kinds of seeds both at

[1] Report of work done while in the Seed Laboratory, United States Department of Agriculture.

the temperatures recommended and at other temperatures, and has verified these conclusions. Duplicates of one hundred seeds each were used in making nearly every germination test. In a few tests duplicates of only fifty or seventy-five seeds each were available, on account of the small size of the samples used.

Method and apparatus

The sweet pea and nasturtium seeds were tested in moist canton flannel, using two thicknesses of the flannel under the seeds and two thicknesses over them. Balsam, California poppy, cosmos, larkspur, marigold, mignonette, pansy, and zinnia seeds were tested between moist blotting papers, two thicknesses above and two below. Candytuft, cypress, petunia, pink, poppy, portulaca, and snapdragon seeds were tested on top of four thicknesses of moist blotting paper. In the tests which were made in 1914 the poppy seeds were tested both between moist blotting papers and on top of moist blotting paper.

All tests were made in standard water-jacketed copper germinating chambers, and were continued until no more seeds or only an occasional one germinated. The progress of germination was carefully watched, and all germinated seeds were counted and thrown away at frequent intervals in the tests which were made in 1912, and each day after germination began in the tests which were made in 1914.

The seeds were tested with the use of the constant temperatures 15°, 17.5°, 20°, 22.5°, 25°, 28°, and 30° C, and with daily alternations of temperature between 20° C. as the lower temperature and 28°, 30°, 31°, 32°, 35°, and 37° as the higher temperatures in the different alternations. The temperatures named as the higher temperatures in the alternations are those indicated by thermometers inserted in the tops of the chambers, and are 1° or 2° C. higher than the highest temperatures reached within the blotters or cloths in which the seeds were being tested. The alternations include some in which the seeds were kept from four to seven hours daily in a chamber which was constantly maintained at the higher temperatures, and the rest of the day in another chamber at the lower temperature; and others in which only one chamber was used,

this chamber being slowly heated during the forenoon, and cooled either slowly or rapidly as desired during the afternoon. When only one chamber was used the heating was accomplished by means of a properly adjusted gas flame below the chamber, and the cooling by means of a graduated stream of cold water in the top of the water jacket.

The species investigated may be divided into two groups: (1) those whose seeds germinate well at any constant temperature from 17.5° to 22.5° C., and also with temperature alternations; (2) those whose seeds require a temperature cooler than 20° C. for complete germination. Some of the samples in each group contained many dead seeds, or seeds incapable of germination at any temperature.

Results

Although a direct comparison between the tests made during the two periods (1912 and 1914) is impossible, the results of all the tests can best be discussed together. They will be considered from three standpoints: (1) the effect of alternating versus constant temperatures; (2) the effect of the different temperatures upon the germinating capacity; and (3) the effect of the different temperatures upon the rapidity of germination.

Alternating versus constant temperatures

All the species included in the investigation, with the possible exception of petunia, germinated as completely and as quickly with a favorable constant temperature as with any alternation of temperatures. It should be remembered, however, that taking the seeds out of the chambers to count those germinated introduced a brief change of temperature which may not have been entirely without effect. The influence of this brief temperature change, if it has any, would be greater when the germinated seeds are counted every day or two as in these experiments, than if they were counted less frequently.[2]

[2] While the use of an alternation of temperatures does not seem to be necessary for satisfactory germination of the kinds of seeds treated in this paper, it is very desirable, and in some cases imperatively demanded, with many other kinds of seeds. This subject will be treated in an article to appear shortly in the *Journal of Agricultural Research*.

It may be convenient in seed testing laboratories to use alternating temperatures in conducting germination tests of some of the kinds of seeds considered, in order to conform to methods established for use in testing the germination of other kinds of seeds. This matter will be discussed later.

GERMINATING CAPACITY

Twelve of the species studied belong in group 1. Table I shows the results, so far as total germination is concerned, of the

TABLE I

GERMINATION OF FLOWER SEEDS OF GROUP I

SEEDS	AVERAGE PERCENTAGES OF GERMINATION										
	First series of tests*						Second series of tests*				
	No. of lots	20° C. 20°–28° 20°–30° 20°–31° 20°–32°	20°–35°	20°–37°	28°	30°	No. of lots	15°	17.5° 20° 22.5° 20°–30°	25°	30°
Balsam	1	98 to 99	98	98	98	....	3	94	94 to 98	97	98
Cal. poppy	2	62 to 70	62	62	59	....	1	72	77 to 85	74	68
Candytuft	2	74 to 80	83	70	74	62	5	79	78 to 80	78	76
Cosmos	1	80 to 85	....	80	....	....	1	....	87 to 92	....	90
Cypress	1	91 to 98	88	88	84	....	2	76	78 to 80	74	78
Marigold	....	.....	....	....	....	....	4	66	62 to 64	62	38
Mignonette	2	68 to 71	72	66	62	....	3	73	66 to 71	65	61
Petunia	2	67 to 74	64	56	70	....	2	76	71 to 76	79	80
Pink	1	90 to 96	90	92	90	....	7	89	88 to 91	87	87
Portulaca	1	86 to 94	83	90	86	87	1	71	74 to 80	75	71
Sweet pea	....	.....	....	....	....	....	2	84	84 to 94	84	84
Zinnia	1	90 to 94	96	91	91	....	2	79	79 to 85	78	74

*The two series of tests were entirely distinct, no lot of seeds was used in both series.

experiments with this group. Each of the twelve species germinated about equally well at any constant temperatures from 17.5° to 22.5° C., and with the temperature alternations 20°–28°, 20°–30°, 20°–31°, and 20°–32° C.

Seeds of balsam, candytuft, cypress, marigold, mignonette, petunia, pink, sweet pea, and zinnia germinated as completely at 15° C. as at warmer temperatures. Seeds of balsam, candytuft, cosmos, cypress, petunia, pink, portulaca, sweet pea, and zinnia germinated as completely at some or all of the constant tempera-

tures warmer than 22.5° C. as at cooler temperatures. Four of these, candytuft, pink, portulaca, and zinnia, germinated somewhat less completely at 30° than at 25° or 28° C. Seeds of balsam, California poppy, candytuft, cosmos, cypress, mignonette, pink, portulaca, sweet pea, and zinnia germinated as completely with one or both of the warm temperature alternations, 20°–35° and 20°–37° C., as with cooler temperatures.

TABLE II

GERMINATION OF SEEDS AT DIFFERENT TEMPERATURES

TEMPERATURE	AVERAGE PERCENTAGES OF GERMINATION								
	LARKSPUR		NASTURTIUM	PANSY		POPPY		SNAPDRAGON	
	1912 (1 lot)	1914 (3 lots)	1914 (2 lots)	1912 (1 lot)	1914 (1 lot)	1912 (3 lots)	1914 (8 lots)	1912 (1 lot)	1914 (3 lots)
Icebox	53	86	.....	.....	.....	.....	72	.....	38
15° C	.....	81	78	.....	81	.....	78	.....	66
17.5°	67	63	78	.....	87	77	75	.....	67
20°	48	28	74	46	78	70	60	48	62
22.5°	.....	1	66	.....	78	.....	49	.....	59
20°–28°	38	.....	.....	48	.....	70	.....	44	.....
20°–35° (cooled rapidly)	34	.....	.....	48	.....	70	.....	52	.....
20°–31°	29	.....	.....	.....	.....	.....	.....	.....	.....
20°–30°	25	8	65	56	74	66	52	46	52
20°–32°	11	.....	.....	45	.....	58	.....	48	.....
20°–30° (cooled slowly)	.....	.....	.....	48	.....	51	.....	48	.....
20°–37°	7	.....	.....	36	.....	44	.....	48	.....
25°	.....	.....	60	.....	62	.....	.....	.....	.....
28°	.....	.....	.....	39	.....	26	.....	36	.....
30°	.....	.....	7	.....	28	.....	6	.....	19

Seeds of larkspur, nasturtium (2 species, 1 sample each), pansy, poppy (a number of species), and snapdragon belong to group 2, which germinated most completely at a temperature cooler than 20° C. In 1914 the larkspur, poppy, and snapdragon seeds were tested in an icebox in which the average temperature was about 8° C., as well as with the temperature conditions used with the other kinds of seed.

Table II shows the average percentages of germination of seeds of group 2 under the different temperature conditions, arranged in the order of increasing average temperature of the germination

blotters, regardless of the highest temperature reached in the different alternations.

Pansy seeds germinated more completely at 17.5° than at 15° C.; larkspur and poppy more completely at 15° than at 17.5°; while with nasturtium and snapdragon seeds there was no difference between these two temperatures. Although the larkspur seeds tested in 1914 germinated even more completely in the icebox than at 15° C., the slowness of germination in the icebox makes the use of so low a temperature undesirable. Furthermore, the difference in total germination in favor of the icebox temperature was only with one lot of seeds, the other two germinating practically the same as at 15° C.

The rather poor samples of pansy and snapdragon seeds which were tested in 1912 germinated more completely with an alternation of temperatures than with a constant temperature of 20° C. These samples were not tested with the cooler constant temperatures which proved most favorable in 1914. The decrease in the average percentage of germination with rise in temperature above the optimum was rapid in the case of larkspur, somewhat slower in the poppy, and slow and gradual in nasturtium, pansy, and snapdragon. The low optimum temperature for germination of larkspur and poppy is reflected in the recognized practice of sowing these seeds in the fall or very early in the spring, when the ground is cold. It is significant, too, as showing the adaptation of the seed to the general physiology and life history of the plant, that poppies fail to make satisfactory growth if started after the advent of warm weather when the soil temperature is above the optimum for germination of poppy seeds. In the case of larkspur and poppy, there was a great deal of variation in the relation of temperature to completeness of germination between even the different lots of the same kind of seeds. These two species will be considered separately in the following pages.

TEMPERATURE REQUIREMENTS FOR GERMINATION OF LARKSPUR SEED.—Fig. 1 shows graphically the contrast in response to different temperatures of two different lots of larkspur seeds, tested simultaneously in 1914. Each of the three lots tested in 1914 germinated much more completely in the icebox than at 17.5° C. Fig. 2 shows the total percentages of germination of one lot of

larkspur seeds with the different temperature conditions used in 1912. The different alternations are arranged from left to right

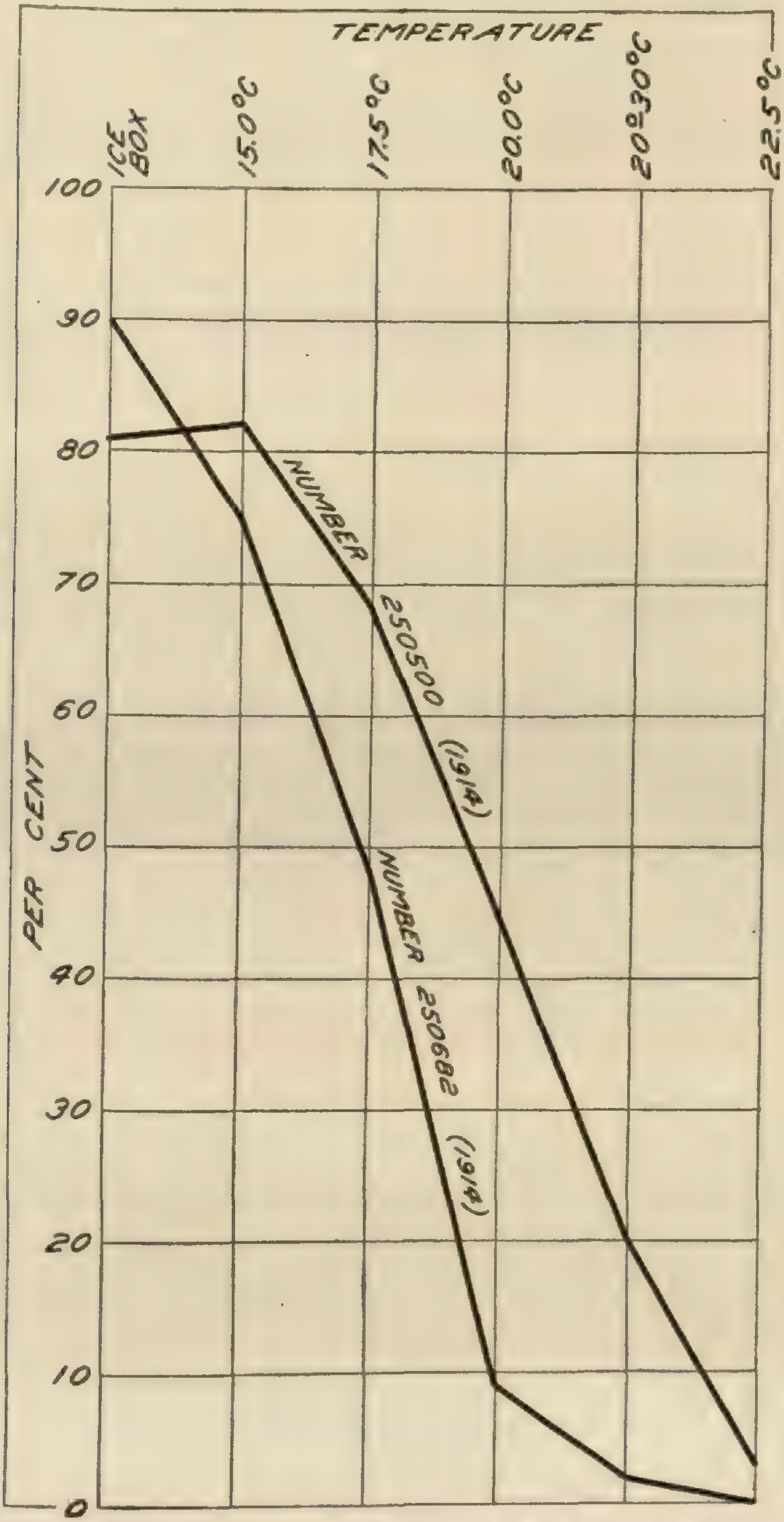


FIG. 1.—Germination of two lots of larkspur seeds

in the order of increasing mean temperature in the blotters. The percentage of germination decreased regularly as the temperature

at which the germination test was made increased from 17.5° to 28° C. The percentage of germination was 14 per cent less in the icebox than at 17.5° C., but greater than at any temperature warmer than 17.5° C. No record was kept of the temperature

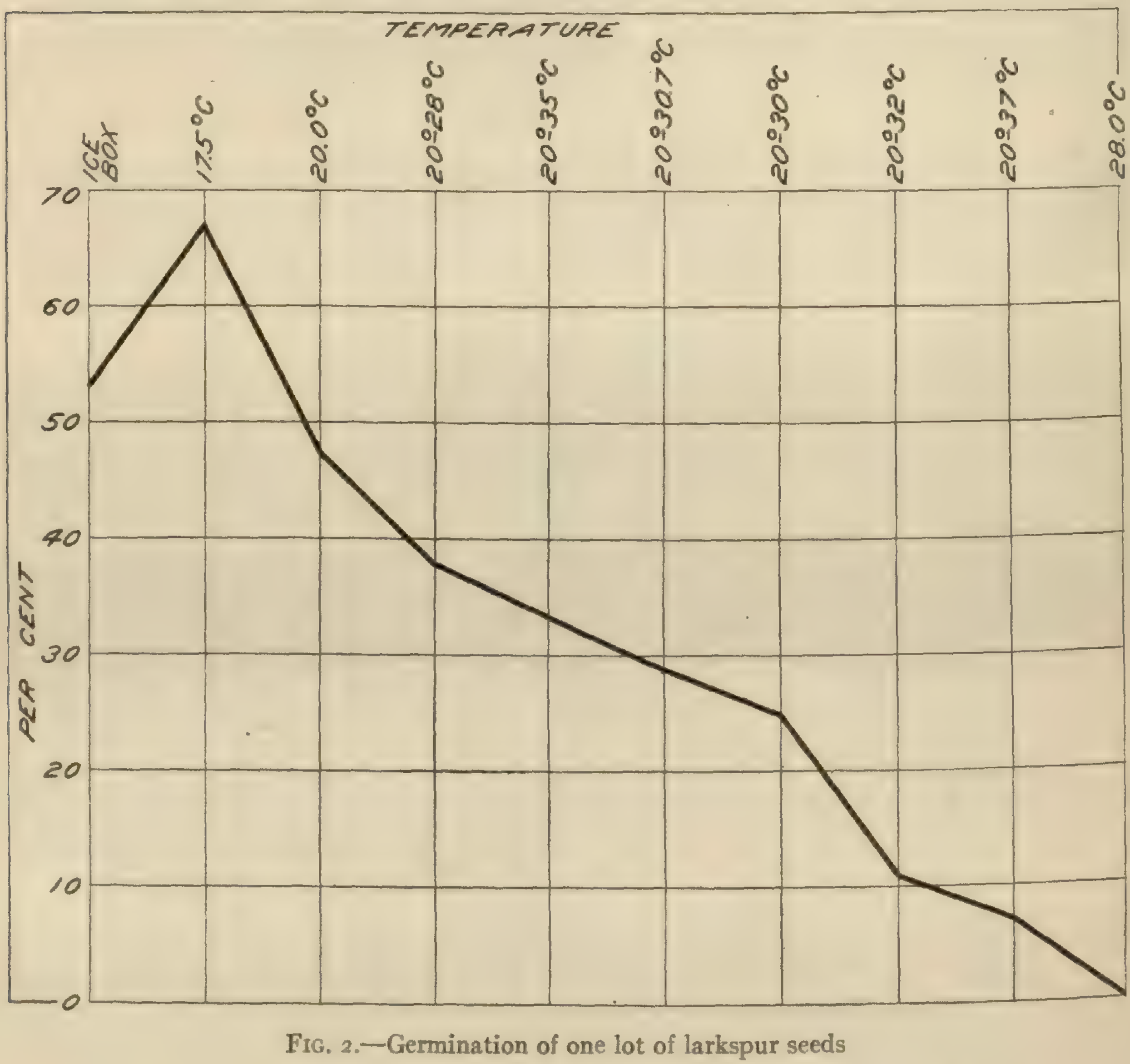


FIG. 2.—Germination of one lot of larkspur seeds

in the icebox, but it was probably cooler than in 1914, when each of the three samples tested showed a higher percentage of germination in the icebox than at 17.5° C.

TEMPERATURE REQUIREMENTS FOR GERMINATION OF POPPY SEED.—One lot of poppy seeds used in the investigations in 1912 was of the opium poppy, *Papaver somniferum*, the other two lots

were not determined as to species. In 1914 three lots of seeds of *Papaver somniferum*, three lots of the horticultural variety "Shirley" of *Papaver rhoeas*, and two lots of an undetermined species were used. Table III gives the percentages of germination of the eight lots which were tested in 1914. In general, the seeds of *Papaver somniferum* were much less sensitive to temperature conditions than were the seeds of the other species. Fig. 3, constructed from the averages in table III, illustrates the relation of temperature to completeness of germination of the three species of *Papaver* included in the tests of 1914. Fig. 4 shows the differences in germination of three lots of seed of *Papaver somniferum* with different temperatures. The temperature alternation 20°–30° C. represents a mean temperature in the blotters of practically 22.5° C. The equivalent value of these two temperature conditions for the germination of larkspur and poppy seeds is evident from the results.

TABLE III

GERMINATION OF DIFFERENT SPECIES OF POPPY AT DIFFERENT TEMPERATURES

Temperature	Percentages of germination										
	Papaver somniferum				Papaver rhoeas var. "Shirley"				Papaver sp.		
	No. 5440	No. 250482	No. 250768	Averages	No. 250224	No. 250693	No. 250752	Averages	No. 250196	No. 250197	Averages
Icebox	55	78	87	73	58	76	76	70	56	88	72
15° C	70	79	94	81	58	88	81	76	62	90	76
17.5°	65	78	88	77	58	78	81	72	61	90	76
20°	66	78	78	74	44	38	36	39	48	90	69
20°–30°	52	76	64	64	38	22	21	27	54	88	71
22.5°	58	78	64	67	28	39	16	28	24	84	54
30°	10	26	6	14	1	0	.2	1	0	1	1

GERMINATION OF POPPY SEED BETWEEN BLOTTERS AND ON TOP OF BLOTTERS.—As previously stated, poppy seeds were tested in 1914 both on top of and between double thicknesses of moist blotting paper. In the icebox the average percentage of germination on top of blotters was sixty-seven, between blotters seventy-three. At 15° C. the average percentages were respectively eighty and seventy-five. In the tests with each of the other temperature

conditions, the average percentages of germination on top of blotters and between blotters differed from each other by less

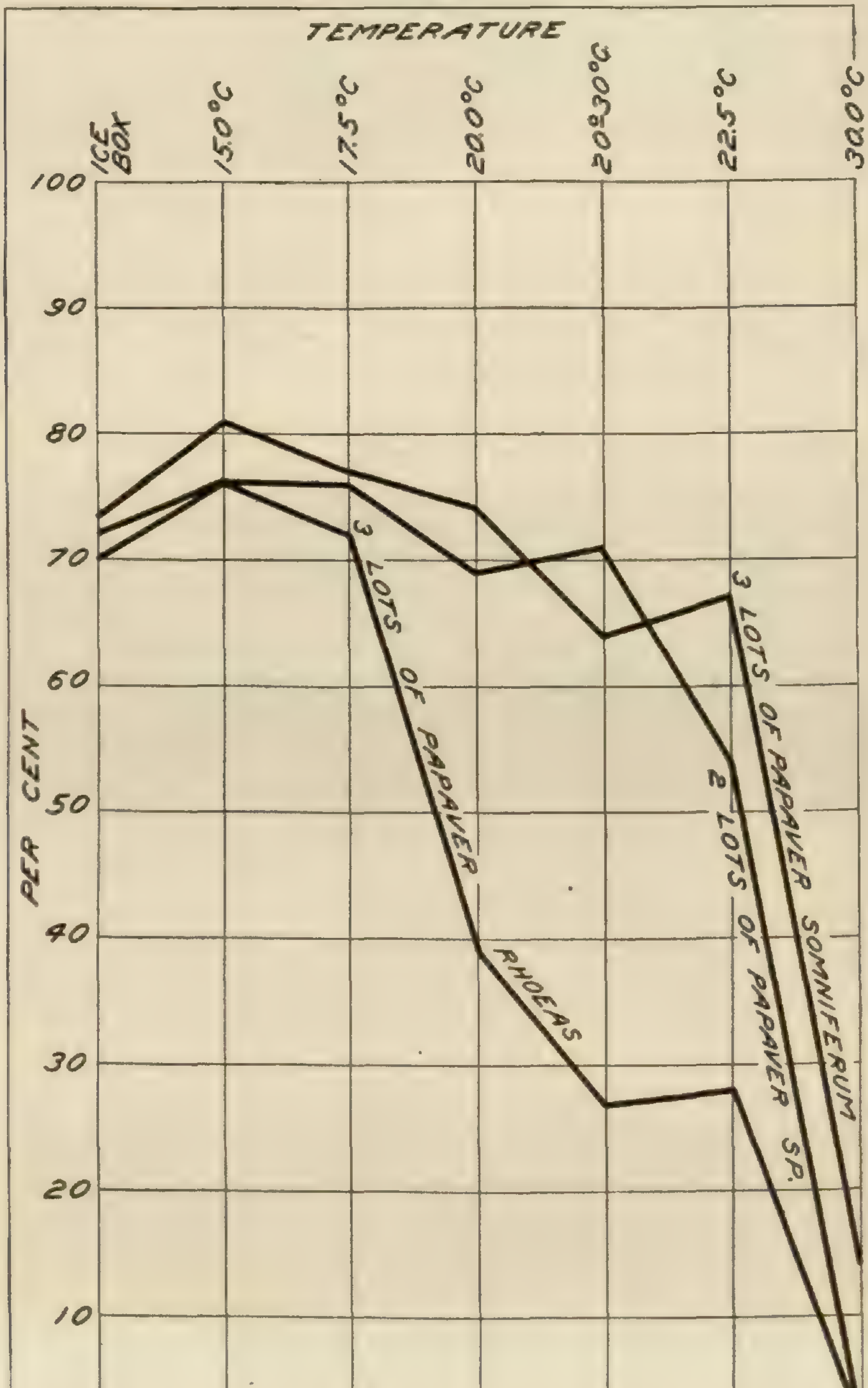


FIG. 3.—Germination of three species of *Papaver*

than 2 per cent. Averaging the results of the fifty-six tests (seven tests of each of eight lots of seeds) on top of blotters, and fifty-six

between blotters, gives fifty-five as the average percentage of germination in each case. There is, then, no advantage in either

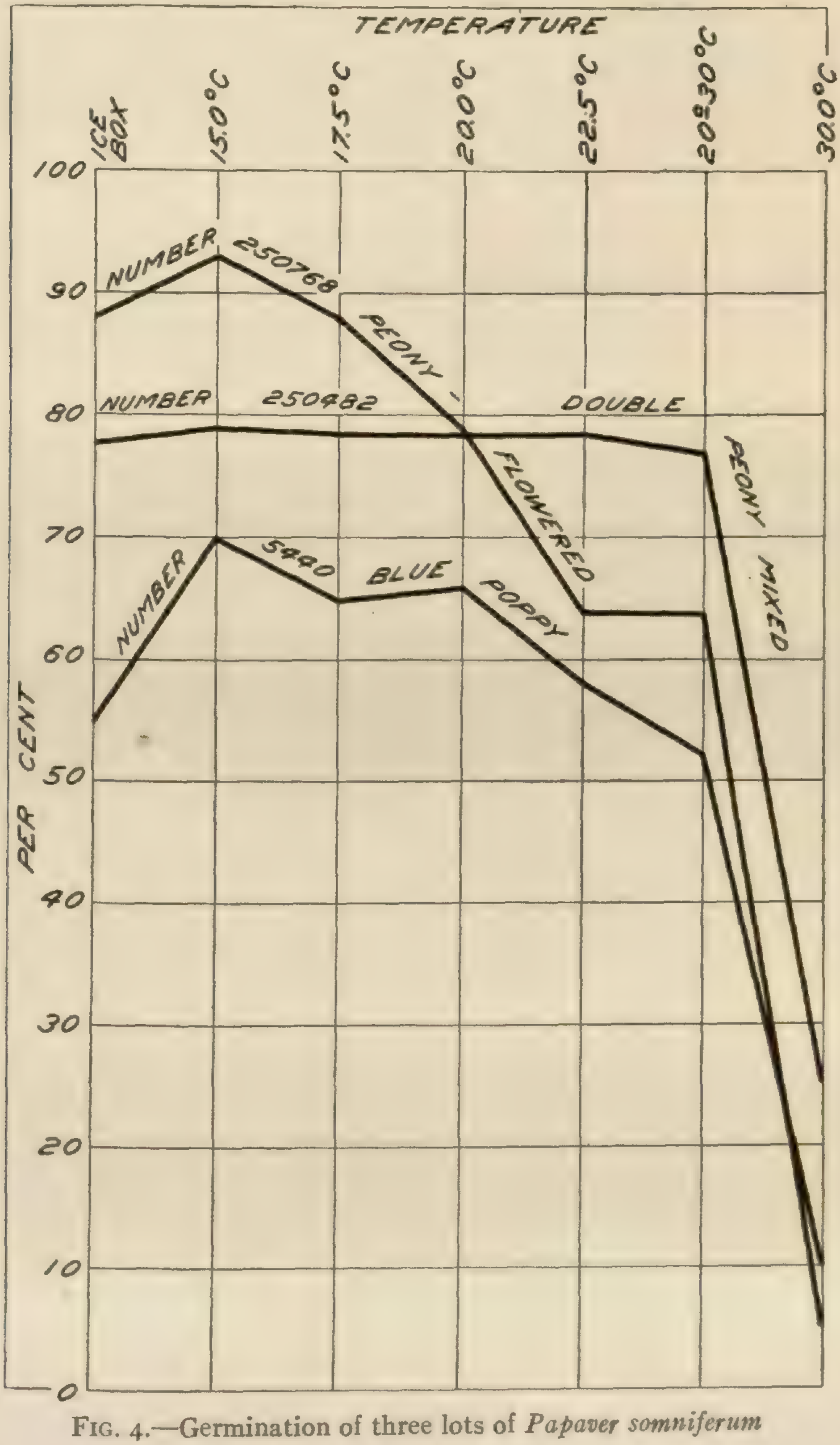


FIG. 4.—Germination of three lots of *Papaver somniferum*

method from the standpoint of completeness of germination. The position of the seeds, however, did affect the rapidity with

which germination took place in the icebox, as will be shown in the following section.

Rapidity of germination

Under favorable temperature conditions, five days were required for germination tests of balsam and cypress seeds; six days for cosmos, marigold, pink, portulaca, and zinnia; eight days for California poppy, candytuft, mignonette, and opium poppy (*Papaver*

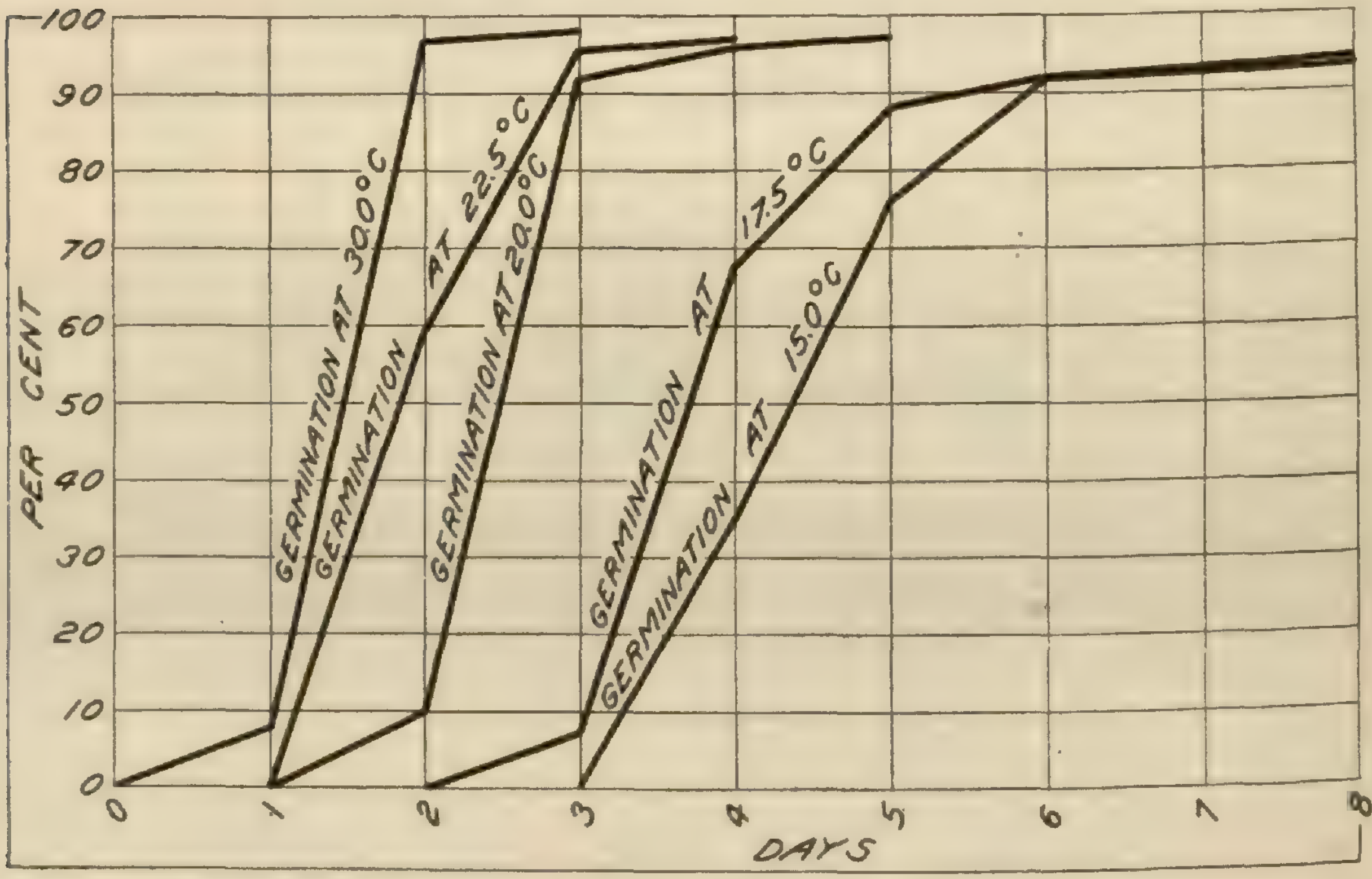


FIG. 5.—Average rate of germination of three lots of balsam seeds

somniferum); ten days for nasturtium, other species of poppy, petunia, snapdragon, and sweet pea; twelve days for pansy; and fifteen days for larkspur. For the germination of strong rapidly germinating lots of seeds, less than the number of days indicated is required. On the other hand, sometimes a very poor lot of seeds or a lot which, although producing vigorous seedlings, germinates slowly, may continue to germinate gradually for a few days longer than indicated. The warmer the temperature within the limit for complete germination, the more rapidly germination took place. A decrease of a few degrees from any given temperature usually retarded germination more than an increase of the

same number of degrees hastened it. This dependence of the rapidity of germination upon temperature was much more marked with some kinds of seeds that with others. Figs. 5–8 show the average rates of germination at different temperatures of a number of different lots each of balsam, cypress, snapdragon, and larkspur

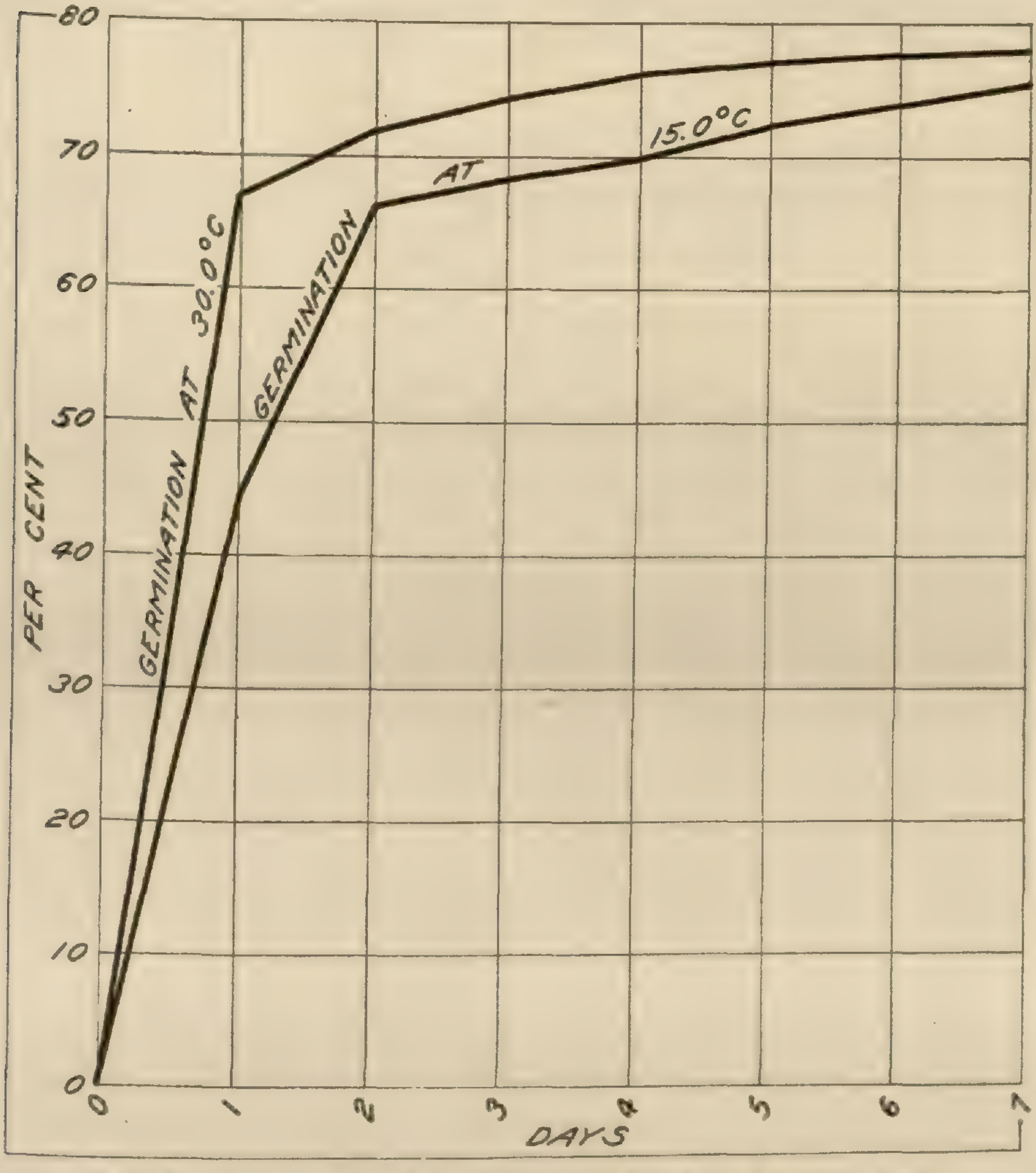


FIG. 6.—Average rate of germination of two lots of cypress seeds

seeds, and illustrate differences in sensitiveness to temperature conditions. Fig. 5, for balsam, is typical also for mignonette, petunia, and portulaca, in so far as the range of temperatures for complete germination is the same. Cypress seeds (fig. 6) germinated more rapidly than any other kind, and almost as rapidly at 15° as at 30° C. Fig. 7, for snapdragon, is typical also for

nasturtium, pansy, and poppy seed. It shows an acceleration of germination by temperatures which were above the maximum for complete germination. In contrast with fig. 7, fig. 8 (for larkspur) shows a retardation of germination as well as a great reduction in total germination by a temperature only 5° C. above the optimum for complete germination.

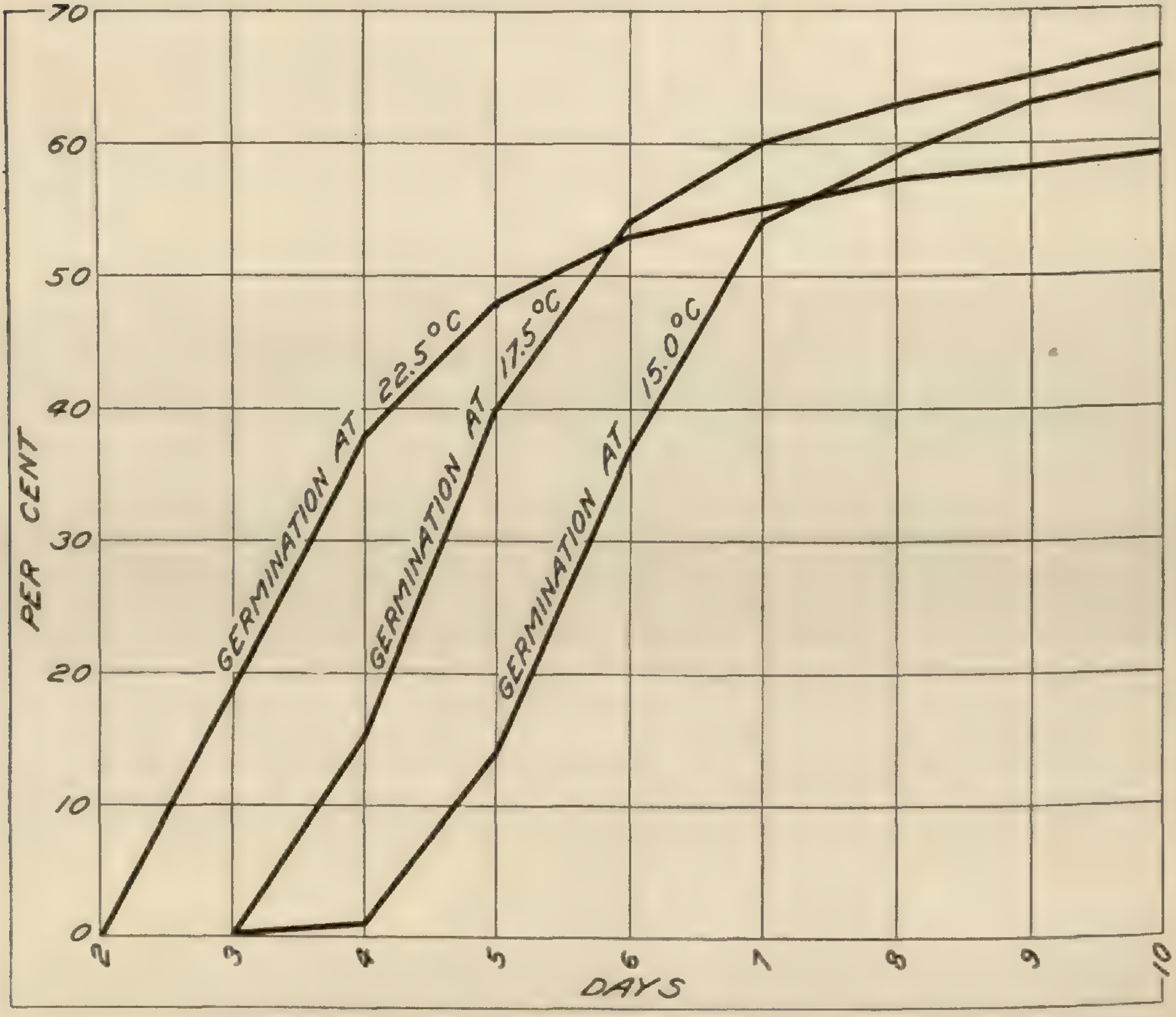


FIG. 7.—Average rate of germination of three lots of snapdragon seeds

The harmful effect of a high temperature on the germination of nasturtium, pansy, poppy, and snapdragon seeds is shown by the fact that all of these seeds germinated more slowly even during the first few days with the temperature alternation 20°–30° C. (not shown in fig. 7, but about equivalent in average temperature to 22.5° C. constant) than at temperatures lower than 22.5° C. Pansy and poppy seeds germinated even less rapidly at 20°–30°

than at 17.5° C. Exposure to 30° for only a few hours each day, therefore, had a retarding effect on germination even during the early days of the test.

A few cases of apparent influence of temperature upon germination require special mention. California poppy seeds germinated much more rapidly and somewhat more completely when the chamber was heated to 30° and allowed to cool very slowly to room temperature than with any of the other conditions of either

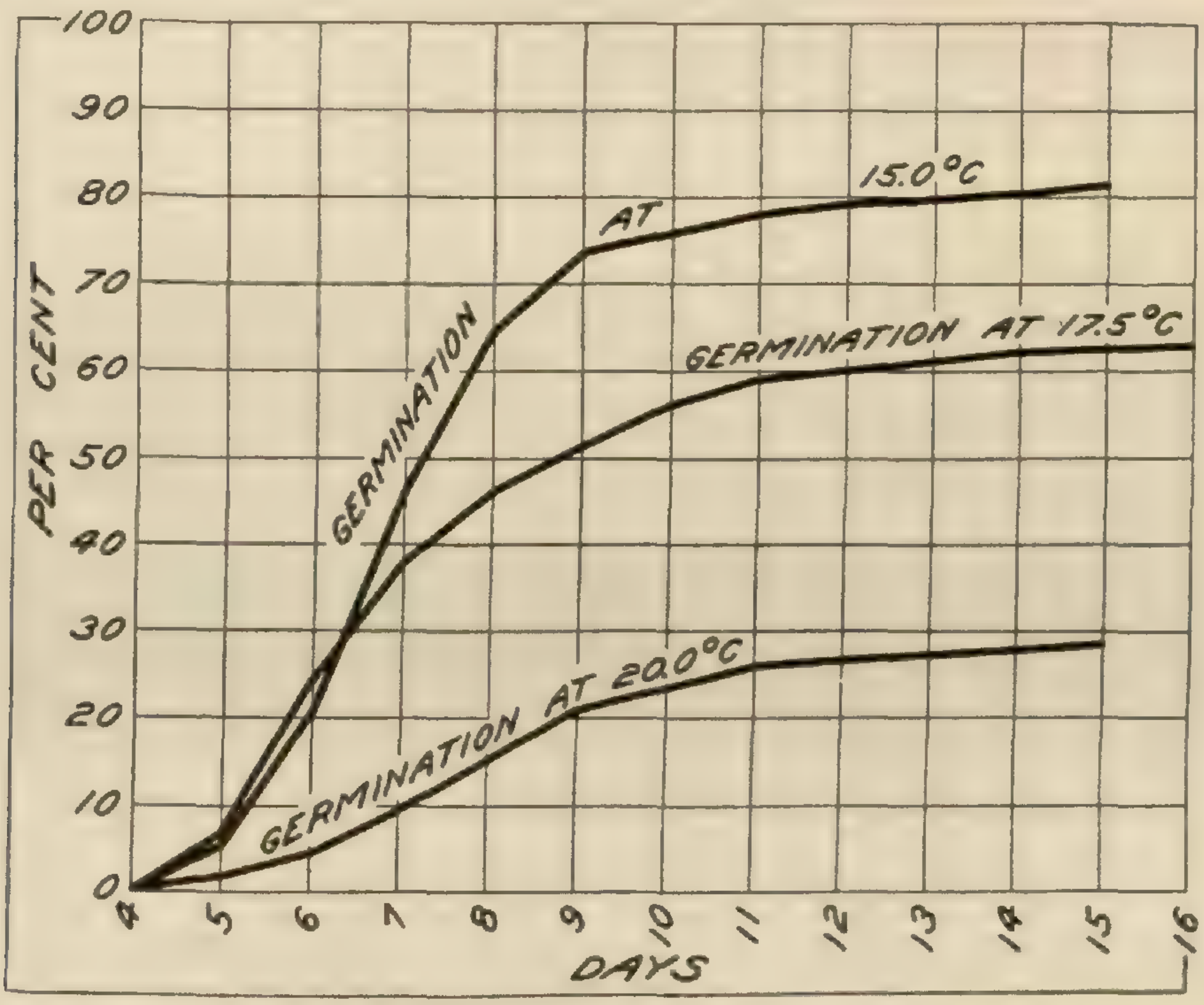


FIG. 8.—Average rate of germination of three lots of larkspur seeds

constant or alternating temperatures used in the series of tests made in 1912.

The cypress seeds were infected with a "damping-off" organism, which destroyed some of the germinated seeds almost as soon as germination began in the tests which were conducted at high temperatures. Some of the nasturtium seeds were badly infected with organisms of decay and with parasitic nematodes, which affected germination more seriously at the higher than at lower temperatures. To avoid difficulties of this kind, so far as possible all sorts of seeds should be tested at temperatures as low as are

consistent with the nature of the seeds in any given case. It is possible also that effective sterilization of the seeds before placing them in the germinator in some cases would alter the conclusions as to optimum temperatures for germination.

The two lots of petunia seeds tested in 1912 germinated somewhat less completely with either of the constant temperatures 20° or 28° than with certain of the alternations of temperatures, especially 20°–30° C. The petunia seeds used in the tests which were made in 1914, however, germinated as completely with any constant temperature from 17.5° to 30° as with the temperature alternation 20°–30° C. In these tests the highest percentage of germination obtained occurred with the constant temperatures 25° and 30°. In testing petunia seeds for germination, probably the most uniformly good results would be obtained with a constant temperature not warmer than 25° or cooler than 22.5°, or with the temperature alternation 20°–30° C.

From 4 to 10 per cent of the sweet pea seeds remained hard at the expiration of the germination tests at different temperatures. No effect of temperature upon the softening and germination of these seeds was noticed.

RAPIDITY OF GERMINATION IN ICEBOX.—In the icebox the first larkspur seeds germinated during the tenth day, the first poppy seeds during the sixth day, and the first snapdragon seeds during the twelfth day. With each kind of seeds, the progress of germination in the icebox was slow. Four weeks were required for a germination test of one lot of larkspur, and three weeks for a germination test of the other two lots of larkspur and some of the lots of poppy. The snapdragon seeds were kept in the icebox over five weeks. At the end of this time germination had practically ceased, but the total percentage of germination (38 per cent) was still but little more than one-half as great as 17.5° C. (67 per cent). The majority of the snapdragon seeds which germinated in the icebox germinated between the twentieth and thirtieth days of the test.

The germination of larkspur no. 250585 began on the eleventh day and was complete in twenty days. At the same time no seeds of larkspur no. 250500 germinated until the nineteenth day,

and germination of this lot continued through the thirty-first day. The rates of germination of these two lots of seeds in the icebox

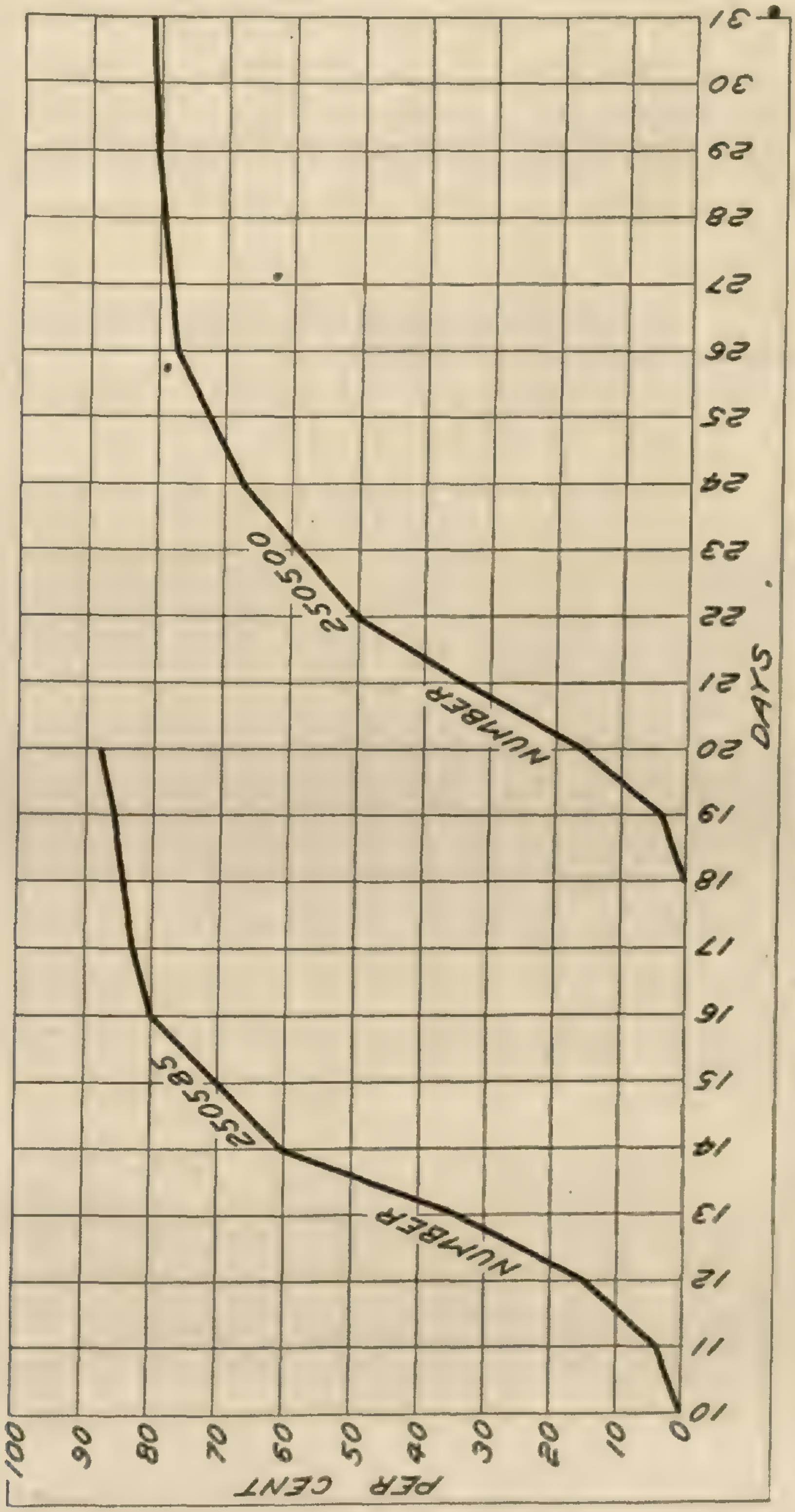


FIG. 9.—Rates of germination of two lots of larkspur seeds in icebox

are shown graphically in fig. 9. Similar but less striking differences occurred in the rates of germination in the icebox of different lots of poppy seeds of each of the three species tested.

RAPIDITY OF GERMINATION BETWEEN AND ON TOP OF BLOTTERS.—In 1914 the poppy seeds were tested simultaneously on top of blotters and between blotters. Except in the icebox, the position of the seeds did not affect the rapidity of germination. In the icebox *Papaver somniferum*, two lots of *P. rhoeas*, and one lot of the undetermined species germinated much more rapidly between blotters than on top of blotters, while there was no difference with the other two lots. The greatest difference was with

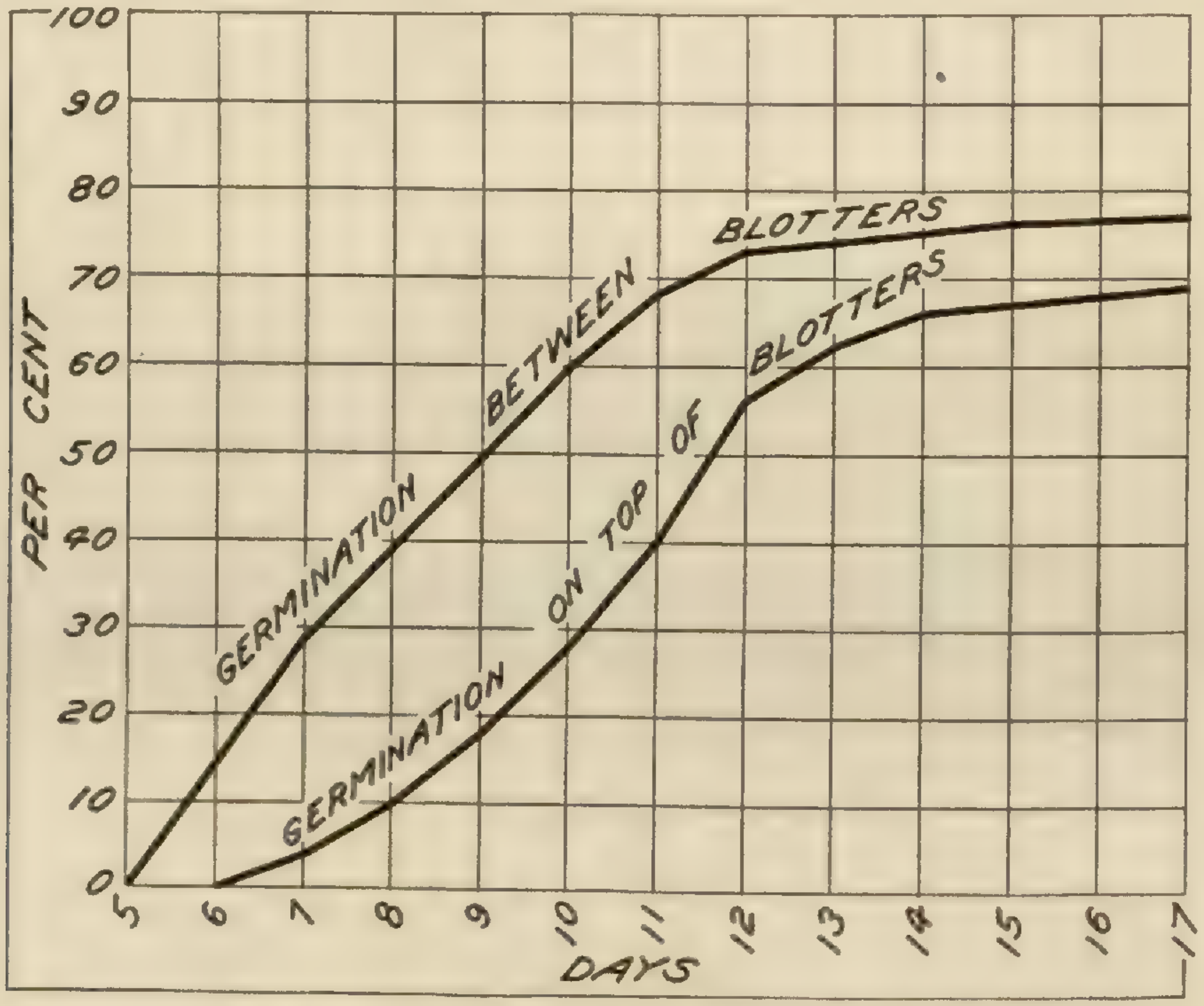


FIG. 10.—Average rates of germination in icebox of six lots of poppy seeds, between blotters and on top of blotters.

one lot of *P. somniferum*, only 5 per cent of which germinated in the first seven days when on top of blotters, in contrast with 51 per cent between blotters. As the temperature of the icebox was very cool, the further reduction of temperature on the surface of the blotters by evaporation probably was sufficient to retard the germination of these lots, and also may explain the lower total germination on top of the blotters. Fig. 10 shows the average rates of germination in the icebox of the six lots of poppy seeds which germinated more rapidly between blotters.

Discussion

It is evident from the foregoing facts that the use of warm temperatures usually increases the rapidity of germination of the species investigated, but that comparatively low temperatures are more favorable for completeness of germination. In conducting germination tests of each species, a temperature should be used which is warm enough to accelerate the progress of germination as much as can safely be done. At the same time, it should not be warm enough to prevent the germination of any viable seeds, or to encourage more than is necessary the development of microorganisms.

When the germination temperature is too warm, frequently the germinated seeds make but little growth, and it is impossible to judge the comparative vigor of different lots of seeds. Sometimes weak seeds of little value will germinate when a warm temperature is used, and will then appear to as good advantage as other strong vigorous seeds. If the germination tests are made with a more favorable temperature, both the strong and the weak seeds will germinate, but in this case the difference will be obvious at once. In this case some seedlings make rapid vigorous growth and are normal in appearance, while others have a watery translucent appearance, grow very slowly, and sometimes have begun to decay before emerging from the seed coat. On the other hand, too cool a temperature decreases the germination, increases the time required, increases also the difference in time required by different lots of seeds of the same species, and thus makes uniform procedure with the different lots impossible. This condition is well illustrated by larkspur samples no. 250500 and no. 250585 (fig. 9).

In conducting germination tests of some of the kinds of seeds, considerable latitude is permissible in deciding upon the temperature to be used. With certain other kinds, as larkspur, the temperature requirements for completeness and rapidity of germination fall within narrow limits.

The substratum should be such as to furnish abundant water to the germinating seeds without limiting the supply of oxygen. For this purpose folded blotting paper well moistened with water

is favorable. Most seeds of medium size can safely be tested between folds of blotting paper. Very small seeds do not hold the separate folds of the blotting paper apart so as to allow circulation of air between them. To insure a sufficient supply of oxygen, such seeds should be tested on top of the moist blotting paper. Candytuft seeds were tested on top of the blotting paper, not because of their size, but because of their mucilaginous covering, which softens when the seeds are wet and sticks the seeds insecurely to both the upper and lower layers of the blotting paper, thus increasing the danger of loss or displacement of the seeds when the blotters are opened to count the germinated seeds. Pansy seeds have a mucilaginous covering similar to the covering of candytuft seeds and may well be tested on top of blotting paper, instead of between blotters as in this investigation. Large seeds, such as sweet pea and nasturtium, should be tested between folds of moist canton flannel or other similar material, instead of in moist blotting paper, because the cloth folds around each seed and supplies moisture to a larger portion of its surface than the blotting paper does.

The seeds should be carefully distributed upon the substratum so that no two seeds touch each other. This guards against the spread of microorganisms, and is of special importance with seeds which are infected with such organisms as those which cause the "damping-off" of seedlings.

Table IV shows the conditions which are recommended for use in making germination tests of the kinds of flower seeds included in the investigation, and the number of days necessary for a preliminary estimate of the germinating capacity and for complete germination. The time allowed for preliminary estimate of each kind is the number of days required for the germination of approximately three-quarters (actual proportions in this investigation varied from 0.7 to 0.9) of the seeds of that kind which are capable of germinating under the conditions indicated. The temperatures given are those which it is thought will give best results with each kind of seeds when both completeness and rapidity of germination are considered. With many lots of seeds germination will be com-

plete in fewer days than are indicated for the completion of the test, and perhaps in exceptional cases a few days longer will be necessary.

Petunia seeds are the only kind for which an alternation of temperatures is recommended, although sweet peas also will germinate as well at 20°–30° C. as with a constant temperature.

TABLE IV

CONDITIONS RECOMMENDED FOR USE IN MAKING GERMINATION TESTS

SEEDS	SUB-STRATUM	TEMPERATURE	NO. OF DAYS FOR	
			Preliminary estimate	Complete test
Balsam	BB*	20° C.	3	5
California poppy	BB	20°	3	8
Candytuft	TB	20°	3	8
Cosmos	BB	20°	3	6
Cypress	TB	20°	2	5
Larkspur	BB	15°	8	15
Marigold	BB	20°	3	6
Mignonette	BB	20°	4	8
Nasturtium	C	17.5°	7	10
Pansy	TB	17.5°	6	12
Petunia	TB	20°–30° or 22.5° C ††	5	10
Pink	TB	20°	2	6
Papaver somniferum	TB	15°	4	8
Other poppies	TB	15°	5	10
Portulaca	TB	20°	3	6
Snapdragon	TB	17.5°	6	10
Sweet pea	C	22.5° or 20°–30° †	5	10
Zinnia	BB	20°	3	6

* Letters used in this column indicate: BB, between blotters; TB, top of blotters; C, cloth (canton flannel).

† Either temperature condition may be used, but 20°–30°C. is probably preferable for petunia.

Petunia seeds will germinate almost as well, and frequently quite as well with the constant temperature 22.5° or 25° C. as with the alternation 20°–30° C., and either of these constant temperatures may be used for approximate results when it is inconvenient to maintain the two temperatures 20° and 30° C. Nasturtium, pansy, and snapdragon seeds will germinate about as completely (although more slowly) with the constant temperature 15° as 17.5° C. These kinds may be tested also at 20° C., although a

lower temperature is somewhat more favorable. Such considerations make it possible to test all the kinds of seeds investigated with approximately optimum conditions by maintaining only three different temperatures. These three temperatures may be either 15°, 20°, and 22.5°, or 15°, 20°, and 30° C., according as petunia and sweet pea seeds are to be tested with a constant temperature (22.5°) or with an alternation of temperatures (20°–30° C.). It should be emphasized, however, that probably more uniformly good results would be obtained by using for each species the temperature indicated in table IV.

GREENWICH, CONN.

SUBTERRANEAN ORGANS OF BOG PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 288

FRED W. EMERSON

(WITH ELEVEN FIGURES)

Introduction

There has been much research on the subterranean organs of plants from many standpoints. The analytical study of these organs as they grow in nature, however, has chiefly been limited to comparatively recent work. In 1899 and 1900 HITCHCOCK (4) published results of work done on the Kansas flora, in which were brief descriptions of the underground parts of a considerable number of native plants, with notes on habitat and length of life. CANNON (1, 2) in 1911 and 1913 added greatly to our knowledge of the behavior of roots in the soil, showing that some of the current ideas have at best been incomplete, and that in the desert there is a wide variation in root behavior. More recently a number of papers have appeared adding information about the root and rhizome systems in a variety of habitats. Among them are papers by HAYDEN (3), MARKLE (6), PULLING (7), and WEAVER (9, 10).

The present paper deals with work carried on in an attempt to discover, first, the exact behavior of the underground parts of plants growing in peat bogs, and to some extent to compare these organs with those of the same species growing in mineral soil; and second, to determine as far as possible the factors involved in any peculiarities in behavior noticed.

While there are many references in the literature to the comparatively shallow roots in swamp lands, it seems that no one has gone into detail in determining just how shallow the roots and rhizomes are, nor with a few exceptions have the biological relationships of these parts been analyzed. YAPP (12) has described some of the relationships of roots and rhizomes in the fen, and SHERFF (8), in his analysis of the subterranean organs in Skokie Marsh,

has gone into somewhat greater detail. In neither of these papers, however, is work reported on the typical peat bog plants.

The main station for this study was Cedar Lake, at Lake Villa, Lake County, Illinois. Supplementary work was carried on in bogs at Miller and Hillside, Indiana, and in a fen at Wolf Lake, Indiana. Cedar Lake is located about five miles south from the Wisconsin line and twenty-two miles west from Lake Michigan. It is situated in the Valparaiso morainic system (5) in a considerable depression in the drift. The western border of the lake is deep and is covered by a floating mat of fibrous peat, while the north and east sides are shallow, and the vegetation passes from the usual hydrophytic forms in the water to shrubs, sedges, and grasses on the shores. This gives opportunity for comparing certain species as they grow in both peat and mineral soils, but with other conditions as nearly the same as is possible to find them in nature. The bog under consideration is of crescent form, fringing the west end of Cedar Lake, and is about 200 m. in width at its widest part. It is composed of a floating mat of peat that is only slightly decomposed, except where it comes in contact with the clay basin at its landward margin. Here it has decayed, forming a hummocky black soil. Judging from its small size, the fact that it has made but a beginning in covering the lake although it is evidently making measurable progress, and the absence of all trees with the exception of a few young tamaracks, it seems evident that this bog is geologically very young. When compared with the vegetation of other bogs of the region, the plant life of this bog is obviously in the very early stages of plant successions, and it is inconceivable that it dates back to glacial times. Thus the supposition that all bogs are relicts from the glacial period seems less plausible.

Field study

In order to determine the exact form and physical relationships of the roots and rhizomes of bog plants, the preliminary work undertaken was the mapping of these parts *in situ*. The organs in question, some of which were very tender, were followed with the finger tips and then laid bare by the removal of all the material above them. Careful measurements were taken and the maps were made

to scale on coordinate ruled paper (figs. 1, 3, 4, 6). In this work it soon became evident that, with the exception of a few species, living plant parts were very rare below a certain comparatively slight depth, and that each species had its own characteristic range of depths. In most cases the roots and rhizomes maintained almost the same level throughout their length. Hence in mapping it was necessary to show only the horizontal arrangement of the organs in question, stating the depth from the surface or the relation to the water table. Since the mat is held up by its own buoyancy, the surface remained at practically the same level

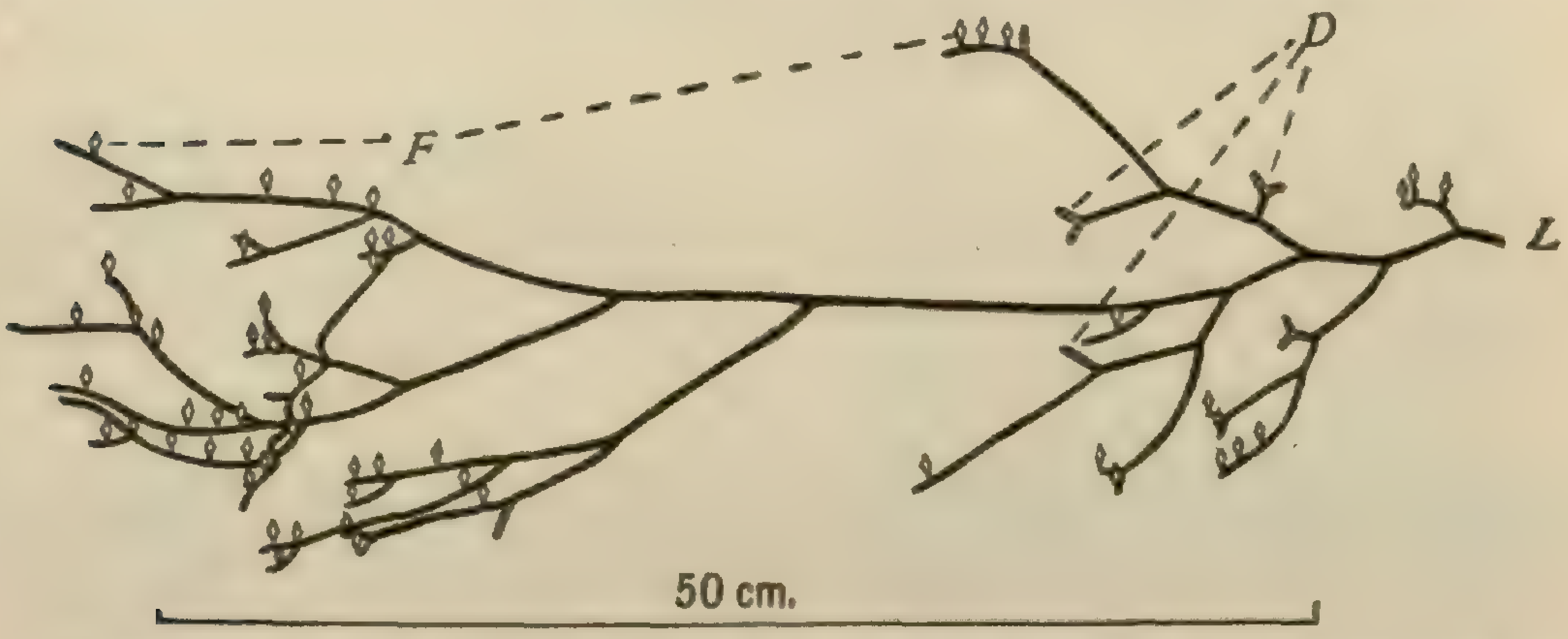


FIG. 1.—*Aspidium Thelypteris*, map of rhizome system: *L*, living attachment to older part of system; *D*, dead tips of rhizomes; *F*, foliage leaves; solid black lines, living rhizomes; note numerous dead rhizome tips, represented by cross lines on white ground, in older parts; dying behind the dichotomous branches leaving them independent is a very common means of multiplication in this fern; depth 4–6 cm.

throughout its extent and throughout the growing season. This distance from soil surface to water table was approximately 6 cm. As may be noted in the maps, the underground parts of bog plants are remarkably straight when compared with those of upland plants. Doubtless this is largely because of the lack of mechanical interference to the growing parts by the spongy peat. The more important species represented in this bog are as follows.

Sphagnum.—This plant grows abundantly over most of the floating mat, especially toward the lakeward margin. It was found to be propagating vegetatively by growing above and dying below. No other means of propagation was found. It appeared to remain alive to a depth of 3–4 cm.

Aspidium Thelypteris.—This species was studied in both bog and swampy mineral soil. The rhizomes were found to be always horizontal. The depth was 2–6 cm. in bog soil, and 1–6 cm. in mineral soil. In no case were living parts found below water. The roots were almost horizontal when near the water table, but nearly vertical and going down to 15–17 cm. deep in a substratum that was only moist. No difference of any sort was apparent in peat and mineral soil. Fig. 1, which is a map of most of the rhizome

FIG. 2.—*Larix laricina* with roots showing horizontal position; inset, young seedling and plant three or four years old showing tap root becoming horizontal; maximum depth 6 cm.

system of one plant, shows that in the older parts there are numerous dead rhizome tips and few leaves, while in the younger parts the plant is vegetating very freely. The older parts were much discolored and too brittle to trace farther than is shown in the map.

Larix laricina (fig. 2).—The larch had no tap root, all the roots being horizontal and above the water level, except where the weight of the tree forced them deeper into the peat. All living roots were 6 cm. or less in depth. There were only a few dozen comparat'vely young larches in the bog in question. No other tree species was found.

Typha latifolia.—The rhizomes of this species assume about the same depth in mineral or peat soil. Those measured varied from 15 to 30 cm. deep in peat, and from 12 to 25 cm. deep in mineral soil. The roots extended diagonally or vertically downward. The deepest extended far below water in both types of soil. A few of the vertical roots were found exceeding 60 cm. in depth. On account of the turbid water they could not well be followed to a greater depth.

Sagittaria latifolia.—This species is not very common in the bog, but is included because it also grows outside of the bog and affords opportunity for comparison in the two habitats. While there is considerable variation in the depth of various parts of a given

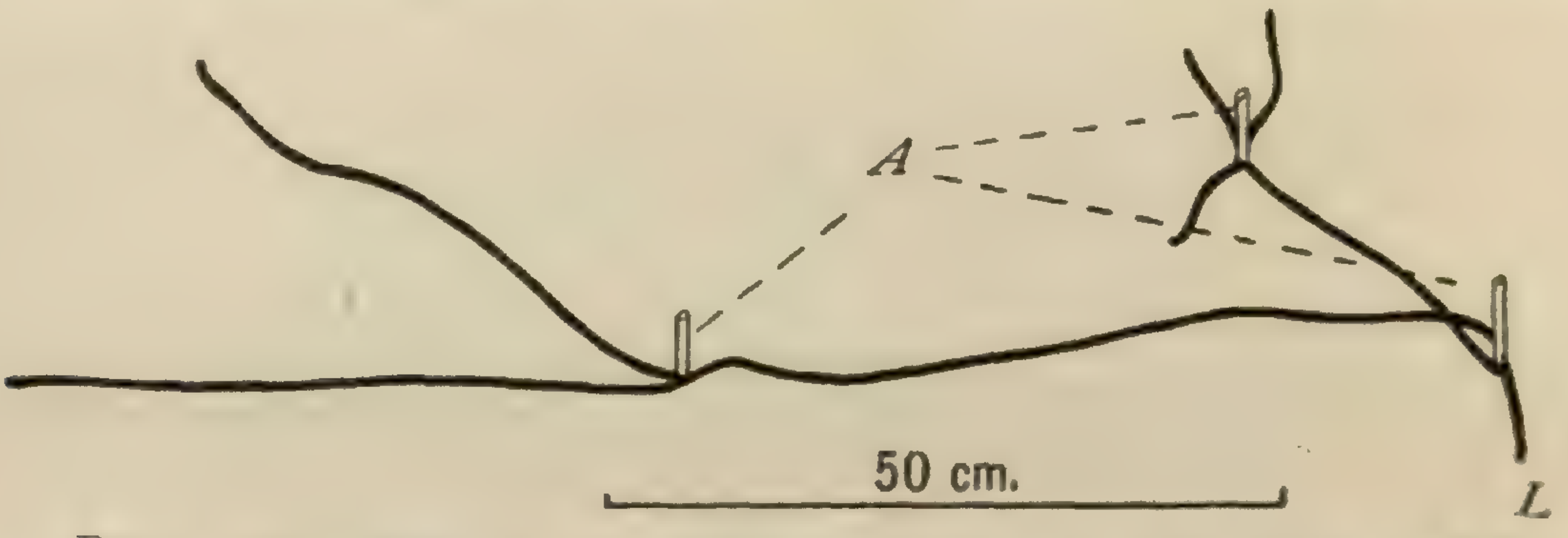


FIG. 3.—*Carex filiformis*, map of rhizome system: *A*, aerial stems; *L*, living attachment to older rhizome; solid black line, living rhizome; depth 8 cm. at aerial stems and going down to 30 cm. between aerial parts.

rhizome system, corresponding parts were found to assume about the same depth in various soils. The aerial parts arise from the rhizome at about 4–6 cm. deep, while at other places the rhizome gradually descends to depths of 10 cm. or more. The root behavior is almost identical with that of *Typha latifolia*.

Scirpus validus.—The rhizomes assumed a depth of 12–15 cm. in all soils where the species was found. A few of the roots extended downward to a depth of 30–40 cm. Nearly all of the roots were vertical, hence the entire subterranean system was below water. This bulrush was fairly common along the lakeward margin of the bog as well as in fens.

Carex filiformis.—This sedge was found in the bog only. Its roots and rhizomes varied in depth from 5 to 30 cm. The roots were approximately horizontal (fig. 3).

Pogonia ophioglossoides (fig. 4).—While this orchid plays no pronounced part in the building of the bog, it is included on account of the peculiar character of its subterranean system. This is made up of a simple but comparatively extensive root system from which the aerial parts grow. This plant has no rhizome, although the root behaves in a manner similar to a rhizome and forms an effective means of vegetative propagation. Branches proper are lacking in the roots, but one or two new roots are likely to arise adventitiously from the base of each aerial shoot. This entire root is 5–6 cm. deep, which is just at the surface of the water in the bogs studied.

Calopogon pulchellus.—In contrast with *Pogonia*, this orchid has very little root system, the chief underground part being a small bulb. The bud of this bulb frequently divides, making two

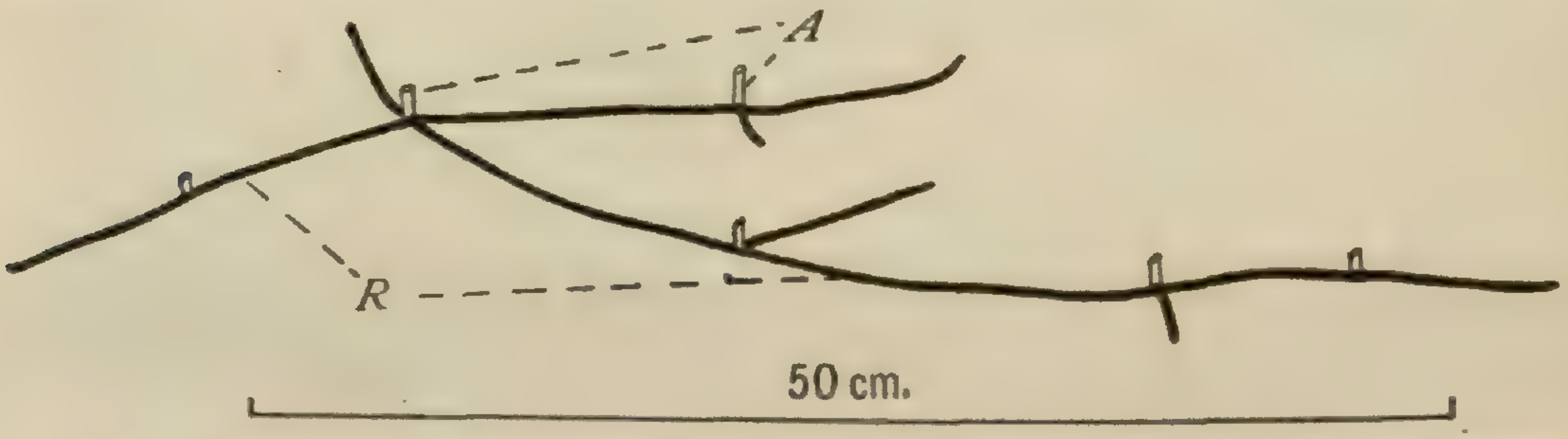


FIG. 4.—*Pogonia ophioglossoides*, map of root system: *A*, aerial stems; *R*, root; plant propagated by roots instead of rhizomes as usual in most bog plants; depth 6 cm.

new plants, which, however, are likely to rema'n attached to each other, hence this is a poor means of disseminating the species. The few simple roots and the bulb structures were found to be dead at about 6 cm. deep, hence no living parts were found below the water.

Betula pumila.—The dwarf birch grows obliquely or vertically upward, putting out roots at various levels in the peat. These roots assume an approximately horizontal position. They were found at various levels from 6 to 18 cm. deep.

Sarracenia purpurea (fig. 5).—This species has a vertical stem, and distorted, usually vertical adventitious roots growing out wherever the stem is covered with peat. All structures die at the water surface.

Drosera rotundifolia.—This plant behaves similarly to *Sarracenia*, except that its living parts do not extend deeper than 2 or

3 cm., and there are not more than three or four feeble, unbranched roots living at any time. Although a comparatively small plant, it appears to be able to grow upward rapidly enough to keep from being covered by the *Sphagnum* in which it often grows.

Lathyrus palustris (fig. 6).—The rhizomes and roots are horizontal in this species, and were not found living below water. The rhizomes were mostly about 5 cm. deep. The roots were few in number, short, and only slightly branched. Those around the aerial stems had numerous large tubercles containing bacteria.

FIG. 5.—*Sarracenia purpurea*, entire root system; stem and roots dead below depth of 6 cm.; roots in normal position.

Decodon verticillatus.—This species is the most prominent pioneer extending the floating mat out over the lake. Wherever the stems come in contact with water, large amounts of cortical aerenchyma form and numerous adventitious roots grow down. Considerable quantities of peat cling to this mass of stems and roots, and thus a floating substratum is formed on which other plants soon begin to grow. Among the most common of these are *Sphagnum*, *Aspidium*, and *Scirpus*. The greatest depth to which the roots of *Decodon* descend in the water was not determined accurately, but it was found that they attain at least a depth of more than 40 cm.

Vaccinium macrocarpon.—Where the prostrate stems of the cranberry come in contact with the moist peat, numerous short adventitious roots appear growing diagonally downward or almost horizontal. As the peat forms above and the roots and stems are weighed down to the water level, they die at the surface of the water. This species, growing with *Aspidium Thelypteris*, forms a tough woody network over a considerable part of this bog.

Menyanthes trifoliata.—The rhizome of this plant assumes an approximately horizontal position from 3 to 9 cm. deep, while the roots may either be horizontal or vertical. The roots were found as much as 12 cm. deep. They were few in number and comparatively short but much branched.

Eupatorium perfoliatum.—The base of the stem of this species assumes an approximately horizontal position near the soil surface

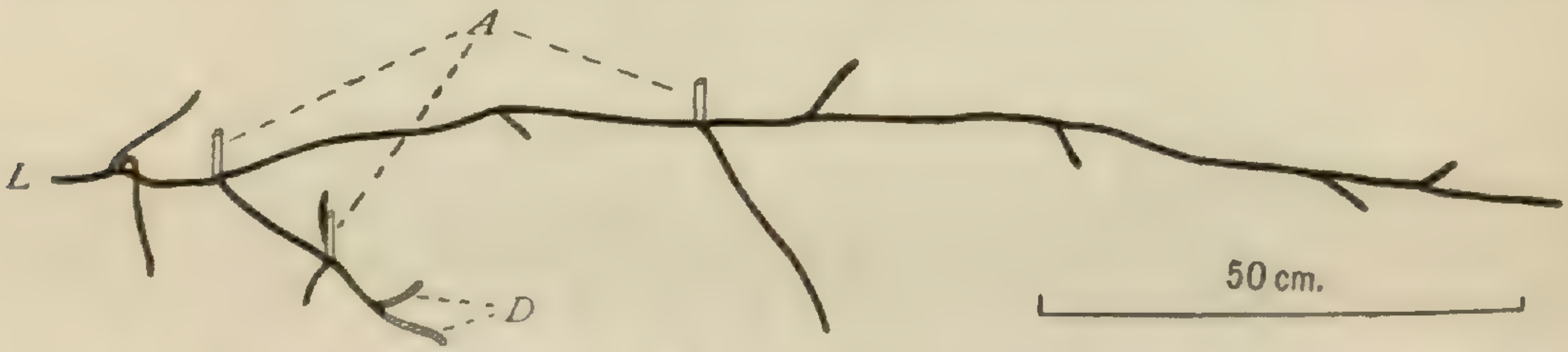


FIG. 6.—*Lathyrus palustris*, map of rhizome system: *L*, living attachment; *A*, aerial stems; *D*, dead rhizome tips; solid black lines, living rhizomes; depth 6 cm.

and the roots grow almost horizontally from this. In the bog the roots were found to reach a depth of 4–6 cm., while in mineral soil where the water table was much lower they reached down to depths of 5–10 cm.

It should be noted that a very high percentage of all the living plant tissue in the bogs studied is above the water level. The part above water usually consists of a mat about 6 cm. thick, made of a coarse feltlike tangle of living roots and rhizomes largely of *Aspidium*, *Carex*, *Vaccinium*, and *Menyanthes*, and often a dense growth of *Sphagnum*. It is difficult to penetrate this tough mat, while just below the water level a sharp contrast appears. Here a fibrous, light brown peat is found in which the dead parts of these same species can often be recognized to considerable depths. Almost the only living parts encountered are occasional roots or rhizomes of *Typha*, *Sagittaria*, *Scirpus*, or *Eriophorum*.

MARKLE (6) working in New Mexico, and WEAVER (9, 10) in the prairies, have noted that two dominant species in an association are not likely to have their roots so placed as to have any marked subterranean competition. This is obviously not the case in the main parts of this bog flora, where two codominants, *Vaccinium macrocarpon* and *Aspidium Thelypteris*, have practically the same level, and together dominate the greater area of the bog. Neither of these two dominant species seems to be overcoming the other. Mingled with these are a number of less important species also at the same level. On the other hand, the deep-rooted forms in which there is no competition are in no case crowding out the shallow rooted species.

Aerenchyma is very common both above and below the water table. Roots and rhizomes which grow below water are all very rich in air tissue, with the exceptions of *Betula pumila* and *Salix* spp. In these species no aerenchyma was found in any of their parts, nor, with the exception of *Decodon verticillatus*, was it found in any woody perennial examined. In most cases herbaceous species have a great deal of aerenchyma.

Tests for H ion concentration in the soils were made by means of the colorometric indicators made by the La Motte Chemical Products Company. The records were made in terms of specific reaction (11). The tests were made in the white porcelain "spot plates," such as are commonly used in the chemical laboratory. By means of pipettes the water to be tested was drawn off from the absorbing parts of the roots. In all cases fruiting or flowering plants were used. Both pipettes and spot plate were thoroughly rinsed in the water to be tested before beginning each test. A series of samples was taken across the bog from the lakeward margin toward the landward side. The water of the lake was uniformly 30 alkaline. The reaction on the floating mat gradually changed from alkaline to neutral, and finally reached 10 acid, 2 or 3 m. from the lakeward margin. This reaction was uniform across the entire mat until the decaying peat was reached on the landward side. Here the acidity decreased until it reached neutrality at a point where the mat was so decomposed that it would no longer bear the weight. It was not possible in any case to discover a difference in

reaction in the water drawn from the immediate surface of the roots and that taken from the peat near by. Hence it seems that all roots in a given area are subject to approximately the same soil reaction. Members of a given species, however, often showed considerable latitude in tolerance to soil reaction. The widest variation found was that of *Larix laricina*. At Mineral Spring, Indiana, in a very old bog in which the peat has become considerably decayed, the reaction about its rootlets was 10 alkaline; at Hillside, Indiana, in a mature bog of fibrous peat, 300–1000 acid; and in the comparatively young mat at Cedar Lake, 10 acid. No marked differences could be seen in subterranean systems of members of the same species growing in peat and mineral soils, or in various natural concentrations of H and OH ions.

Other species which showed a narrower range of tolerance to reaction follow, with the extremes of reaction found in each. Water squeezed from *Sphagnum* had a specific acidity of 100 to 1000; *Aspidium Thelypteris*, *Scirpus validus*, and *Betula pumila* all varied from 10 alkaline to 10 acid; *Sarracenia purpurea*, *Drosera rotundifolia*, and *Vaccinium macrocarpon* varied from neutral to 300 acid. The peat about the roots of *Decodon verticillatus* at the margin of the mat was approximately neutral, while the lake water into which this species was migrating was 30 alkaline.

Experimentation

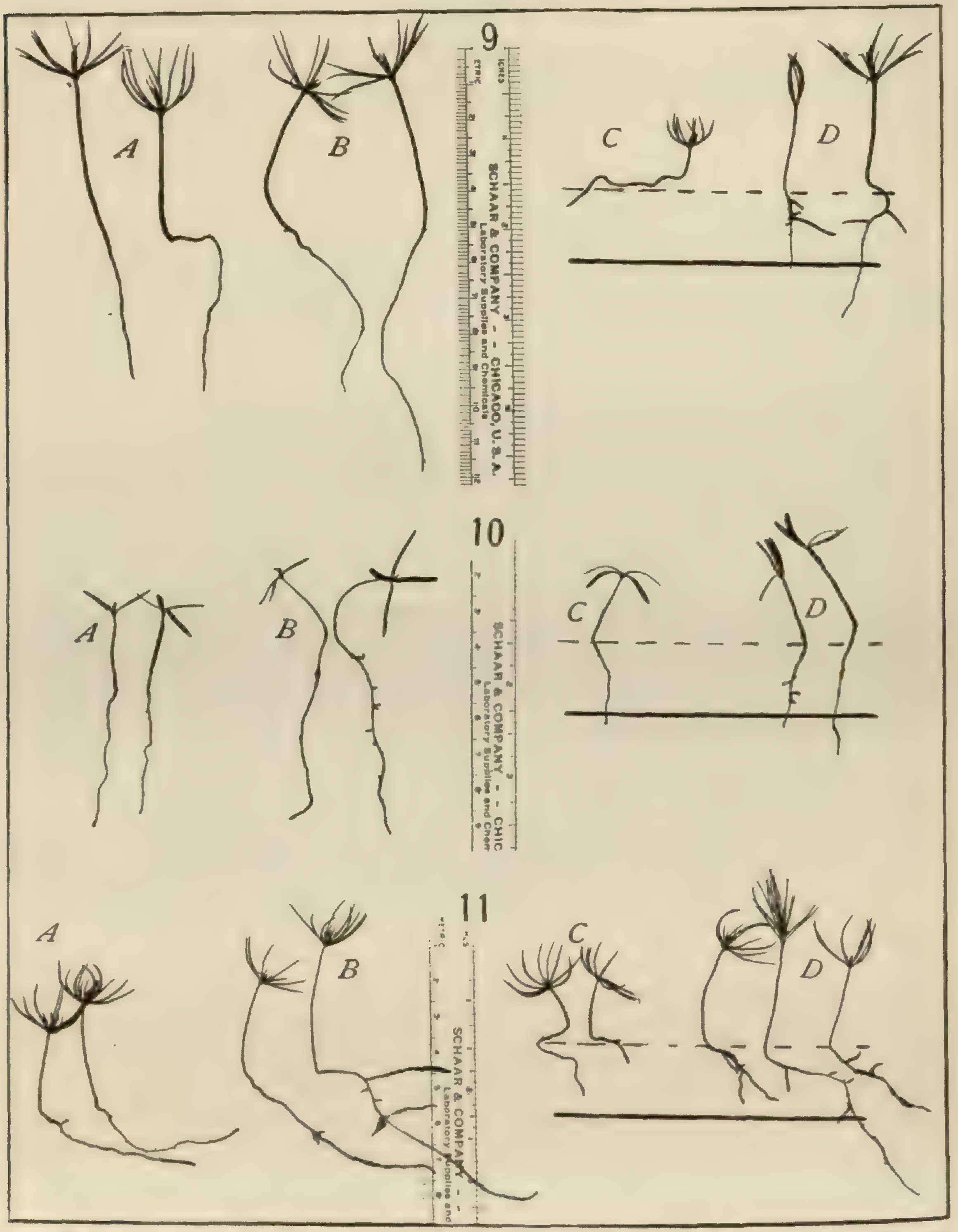
In order to determine the factors involved in the horizontal placing of roots in bogs, the following experiment was carried out. Galvanized iron boxes, 10 cm. × 15 cm. × 55 cm., were made with the bottom and one side replaced by a diagonal pane of glass. This glass was covered on the outside by a piece of galvanized iron which could readily be removed, making it easy to make observations of the roots, but at the same time keeping them protected from the light except during examination. In certain parts of this experiment, as indicated later, a fixed water table was maintained by keeping the boxes in pans of water of proper depth (figs. 7, 8). All metal surfaces were given two coats of Acme asphalt varnish. Various germinating seeds were planted in these boxes. The most

significant results are shown in figs. 9–11. Distilled water was used throughout the experiment for watering the seedlings and maintaining the water level. This distilled water had a reaction of approximately 1 (neutral), although in some cases it showed a slight trace of acidity from carbonic acid. All the peat maintained

FIGS. 7, 8.—Growing boxes used in experimental work: fig. 7, growing box with diagonal glass side facing camera (removable sheet iron false side is in place covering glass); fig. 8, growing box similar to that in fig. 7 placed in pan containing water, thus maintaining fixed water level in growing box.

a specific acidity of 3 throughout the experiment. The garden soils varied from specific reaction 1 to specific alkalinity 10. This last reaction was reached near the end of the experiment in the wet garden soil. The experiment was carried on for about two months during February, March, and April, 1921, in the cool room of the greenhouse. At the end of this period the plants were removed

from the soil and characteristic specimens, showing as far as possible the extremes of size and form, were photographed.



FIGS. 9–11.—*A*, seedlings grown in moist garden soil; *B*, in moist brown fibrous peat; *C*, in garden soil in which water level was maintained at approximately 2 cm. below soil surface; *D*, in brown fibrous peat in which water level was kept at about 2 cm. below soil surface; broken line shows location of soil surface, solid line of water surface; fig. 9, *Pinus Strobus;* fig. 10, *Abies balsamea;* fig. 11, *Picea excelsa*.

1. *Pinus Strobus* (fig. 9 *A*, *B*).—In both moist garden soil and moist peat the tap root assumed approximately the vertical position,

deviation from this direction being chiefly from the interference of the diagonal glass side of the growing box. It is also noteworthy that in both of these cases laterals were lacking. In garden soil in which a water table was kept, capillarity caused the water to rise until the soil was practically saturated to the surface. Only one pine seedling lived throughout this part of the experiment. A number of seedlings started to grow, however, and all behaved in the same way. All the tap roots showed a slight tendency to penetrate the soil, but the plants soon fell over and the roots grew in an approximately horizontal direction, producing no laterals (fig. 9 *C*). Those growing in peat in which a water level was kept behaved in a still different manner. On account of the fibrous spongy structure of the peat, aeration was possible to the water surface. The tap roots grew downward about as in the moist garden soil and peat, although somewhat less rapidly. The most obvious difference began to appear when the tips of the tap roots reached the water level, when growth almost completely ceased. In a few cases the roots continued to grow slowly for some time after reaching the water level, but in all cases these longer roots died back to about the surface of the water. Strong laterals always appeared and took approximately the horizontal position. The study of this species was suggested by the statement made by PULLING (7) that *Pinus Strobus* has "a deep rigid root habit." In examining the root systems of this tree at various ages in the Hillside bog and at Mineral Spring no root was found extending more than a few centimeters deep and all were horizontal. From this experiment and from field observation it is obvious that the tap root is ephemeral under bog conditions, and that very shallow horizontal laterals make up the entire root system.

2. *Abies balsamea* (fig. 10).—Under all the conditions of this experiment the roots grew downward and all were putting out laterals at the end of the experiment. At the water surface the roots behaved as in the case of *Pinus Strobus*.

3. *Picea excelsa* (fig. 11).—Throughout there was evident a decided tendency for the roots to assume an almost horizontal position. In some cases the roots penetrated somewhat below the water surface without showing any ill effects, although the rate of growth was greatly checked on entering the water.

Discussion

A comparison of the action of the roots of seedlings under experimental control with observations in the field shows that there are four general types of behavior of subterranean organs in these bogs.

1. The roots and rhizomes assume an approximately horizontal position above the water table. Typical forms are *Aspidium Thelypteris*, *Picea excelsa*, *Larix laricina* (fig. 2), *Carex filiformis*, *Pogonia ophioglossoides*, *Potentilla palustris*, *Lathyrus palustris*, and *Vaccinium macrocarpon*.

2. The tap roots of the seedlings die at the water surface and horizontal laterals appear above. Examples are *Pinus Strobus* and *Abies balsamea*.

3. All underground parts are approximately vertical and die near the water surface, usually with non-horizontal laterals or adventitious roots appearing above, as in *Sphagnum*, *Calopogon pulchellus*, *Sarracenia purpurea* (fig. 5), and *Drosera rotundifolia*.

4. The rhizomes and roots are able to grow under water. Important species are *Typha latifolia*, *Sagittaria latifolia*, *Scirpus validus*, *Eriophorum*, *Betula pumila*, and *Decodon verticillatus*.

While doubtless there are many factors influencing the location of roots and rhizomes in bog soils, it becomes evident that the two most potent are hereditary tendencies and water level. Rhizomes of certain plants, such as *Typha*, assume a depth apparently determined by heredity, which places them below the surface of the water. Such plants as can readily endure submergence are able to persist in the bog unless other factors interfere. Obligate deep-rooted plants which are intolerant of submergence are eliminated by water from this flora. Rhizomes of certain other species, as for example *Aspidium Thelypteris*, are in all cases superficial, thus permitting their development above water. On the other hand, the roots especially seem to respond rather readily to the water surface. A number of species have only shallow roots where the water table is high, but deeper ones in most peat or moist mineral soil. This was found to be especially well illustrated by *Aspidium Thelypteris*, *Pinus Strobus*, and *Acer rubrum;* hence it is apparently not the quality of the soil but the presence of water

that induces shallowness. Doubtless the lack of oxygen plays a considerable part in checking growth under water. It is possible that bog toxins may in part be responsible for the poor development or in some cases even the death of the roots of certain species.

Summary

1. Subterranean systems of plants growing on floating mats were found to be very superficial, nearly all the living tissue being above the level of the water.

2. No evidence was found to suggest that acidity or toxins are involved in the shallowness of these organs. Water level was apparently the important factor, aside from hereditary tendencies in certain species.

3. Roots of codominants were in close competition without apparent damage resulting to them.

4. Three types of behavior were noted, resulting in the superficial placing of the living parts of bog plants: (*a*) the parts assume the horizontal position above the water level; (*b*) the tap root is ephemeral in the bog and is replaced by horizontal laterals; (*c*) the roots are all vertical and die at the water surface.

5. Certain plant parts were found to be able to thrive under the water in the bogs.

6. There is no apparent marked difference in the subterranean organs of a given species growing in a bog and in comparable conditions in mineral soils.

I wish to express my appreciation to Dr. H. C. Cowles and Dr. Geo. D. Fuller for encouragement and helpful suggestions during the progress of this work.

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MORPHOLOGICAL STUDY OF CARYA ALBA AND JUGLANS NIGRA

THEO. HOLM

(WITH PLATES XV, XVI, AND ONE FIGURE)

The systematic position of the Juglandaceae has been somewhat disputed, some workers having referred the family to the close vicinity of the Anacardiaceae, although the floral structure is very different, and the resiniferous ducts so characteristic of these are totally absent from the Juglandaceae. Since the floral structure has been incorrectly explained in American manuals, it is thought advisable to redescribe this. Moreover, there are several points with regard to the internal structure and the germination which may be of interest to the student of plant morphology, besides that the American representatives are very little known from this particular point of view.

Flower

According to EICHLER,[1] the staminate flower of *Carya* (fig. 1) consists of two prophylla (*P*), which grow together with the subtending bract (*L*), thus forming a three-lobed involucre (figs. 2, 3) suggesting that of *Carpinus;* there is no perianth. The stamens, two to ten, have very short filaments, and are free (fig. 4). In the pistillate flower (figs. 5, 6) the bract is much longer than the two prophylla and the single, or very seldom two perianth-leaves (figs. 7, 8). In other words, the staminate flower has a three-lobed involucre, but no perianth; on the other hand, the pistillate has a very rudimentary perianth, consisting of a single leaf, or very seldom of two minute leaves.

This simple and natural explanation of the floral structure, however, has been ignored or completely misunderstood by subsequent writers in this country. It is strange to see the incorrect description that has been given in the treatments of the North

[1] EICHLER, A. W., Blüthendiagramme. **2**:32. 1875.

American flora. For instance, in GRAY'S *New manual of botany*[2] the staminate flower of the Juglandaceae is said to have "an irregular calyx adnate to the bract," and the pistillate flower to have "a regular 3–5-lobed calyx adherent to the ovary." Furthermore, under *Carya* the staminate flower is simply described as "stamens 3–10; filaments short or none, free," while "a four-toothed calyx; petals none" is attributed to the pistillate flower.

SARGENT[3] describes the staminate flower of *Carya* as follows: "Calyx usually 2-, rarely 3-lobed, subtended by an ovate acute elongated bract free nearly to the base, and usually longer than the ovate rounded calyx-lobes." In the pistillate flower the calyx is said to be "reduced to a single posterior lobe," and the ovary to be "inclosed in a perianth-like slightly 4-ridged involucre, composed by the more or less complete union of an anterior bract and 2 lateral bractlets, adnate below to the ovary, unequally 4-lobed at the apex."

BRITTON[4] describes the staminate flower of Juglandales as "consisting of 3–numerous stamens with or without an irregularly lobed perianth adnate to the bractlet," and the pistillate "bracted and usually 2-bracteolate with a 3–5-lobed (normally 4-lobed) calyx or with both calyx and petals." Under *Hicoria* the staminate flower is said to possess "a calyx adnate to the bract, 2–3-lobed or 2–3-cleft," and the pistillate flower is described as "bract fugacious or none; calyx 4-toothed; petals none." This same description is reprinted in the second edition of BRITTON and BROWN'S *Illustrated flora*.

By SMALL[5] the staminate flower of the Juglandales is said to possess "a 2–6-lobed calyx bearing several rows of stamens, or the calyx obsolete," while the pistillate flower is described as "consisting of an involucrate incompletely 2–4-celled gynaecium: calyx partially adnate to the gynaecium." Under *Hicoria* the staminate

[2] ROBINSON and FERNALD, A handbook of the flowering plants and ferns of the central and northeastern United States and adjacent Canada. p. 330. 1908.

[3] SARGENT, C. S., The Silva of North America. 7: 1895.

[4] BRITTON, N. L., Manual of the flora of the Northern States and Canada. 3d. ed. p. 322. 1907.

[5] SMALL, J. K., Flora of the southeastern United States. 2d. ed. p. 332. 1913.

flower is described as "a 3-lobed calyx," and the pistillate as "a calyx of 1 sepal adnate on the ovary."

With respect to the flowers of *Juglans*, EICHLER describes the staminate flower (fig. 10) as consisting of two prophylla (*P*), which with the two to five perianth leaves grow together with the subtending bract; the six to forty stamens have very short, free filaments. The pistillate flower has a superior, four-leaved perianth; the ovary, bract, and prophylla all unite together, their edge being visible as an indented line below the perianth (fig. 13). The staminate flower, therefore, has two prophylla and a two to four-leaved perianth, which grow together with the subtending bract; the pistillate has a superior perianth of four leaves, and the subtending bract beside the two prophylla grow together with the ovary.

As was the case of *Carya*, this very simple structure has been completely misunderstood by subsequent writers in this country. ROBINSON and FERNALD do not describe the staminate flower of *Juglans* in any other way than "stamens 12–40; filaments free, very short." On the other hand, the pistillate flower is said to possess "a four-toothed calyx, bearing four small petals at the sinuses."

SARGENT attributes "a perianth sessile or pedicellate, three to six-lobed in the axil of an adnate to an ovate acute bract free only at the apex" to the staminate flowers. The pistillate flower is described as being invested by a villous involucre adnate to the ovary, and formed by the union of the anterior bract, sometimes free nearly to the base, and two lateral bractlets free only at the apex, and variously cut into a laciniate border shorter than the erect lanceolate calyx lobes inserted at the summit of the ovary.

By BRITTON the staminate flower of *Juglans* is said to have a "perianth 3–6-lobed," and the pistillate "calyx 4-lobed, with 4 small petals adnate to the ovary at the sinuses." SMALL describes the staminate flower in the same manner, while the pistillate is said to have "the sepals adnate to the ovary."

In "*Flora of the District of Columbia and vicinity*," published under the auspices of the Smithsonian Institution (1919), no

description is given of the floral structure, except that the fruit is "a nut inclosed in a shuck or husk, the meat or embryo 4-lobed."

Carya alba

Root

The primary structure may be studied from the thin lateral roots of the seedling. No secondary increase takes place during the first season; thus the epidermis and cortical parenchyma remain intact. The latter consists of about ten compact strata, and the endodermis is very thick-walled, representing a U-endodermis. A thin-walled pericambium of a single layer surrounds the pentarch stele, in which thick-walled conjunctive tissue is much in evidence, surrounding the vessels, and as a narrow group in the center of the stele. On the other hand, increase in thickness is readily noticeable in the primary root of the seedling in its second year. In this the epidermis and the primary cortex have become thrown off, replaced by many layers of homogeneous, thin-walled cork of pericambial origin. Inside the cork is a narrow zone of thin-walled parenchyma, which surrounds a circular band of small strands of stereome (fig. 9, *St*), supporting the leptome (*L*) of the secondary mestome strands. There is now a continuous ring of cambium, from which the secondary mestome is developed, and the thickness of the root depends largely upon the presence of a very broad, central, thin-walled parenchyma, a true pith, containing starch in abundance, but no crystals.

The development of stereome in the root deserves attention, since, so far as known, this tissue does not appear to be commonly represented in roots. In *Carya* it is a secondary structure, which seems to be the general case wherever it occurs in roots. As a primary structure the stereome is extremely rare, known only in a very few genera, *Dirca*, *Anona*, *Celtis*, etc., where it is developed in the primary leptome.

Stem

The apical internode of the seedling is densely covered with hairs of different types, unicellular, long, pointed, which are either single or developed in tufts; and large, sessile, pluricellular, glandular of peltate shape. The cuticle is smooth and the epidermis

is quite thick-walled. The cortex is differentiated into a peripheral sheath of collenchyma, three or four strata, and an interior of thin-walled parenchyma, five to six layers. Rhombic crystals of calcium oxalate were observed in the collenchyma, while aggregated crystals occurred sparingly in the inner part of the cortex. The phellogen arises in the hypodermal stratum of the collenchyma. A thin-walled, starch-bearing endodermis surrounds a band of small isolated strands of stereome, separated from each other by narrow rays of parenchyma. The stele shows a continuous zone of leptome, cambium, and hadrome in deep rays, accompanied by many layers of libriform. A homogeneous, slightly thick-walled pith, destitute of starch, occupies the central portion of the stele; the pith is not septate.

In branches of the mature tree the cork appears in many thin-walled strata; the stereome is well represented as several, until seven, concentric bands of isolated strands, the result of one season's growth. Large rhombic crystals abound in the leptome, and the hadrome is divided by broad tangential bands of moderately thickened libriform. The very thick-walled, porous vessels so characteristic of *Juglans* do not occur in *Carya*, and the pith is nowhere septate.

Leaf

Viewed in superficial sections the ventral epidermis shows the lateral cell walls prominently undulate, hairs and stomata being absent. In the dorsal epidermis the lateral walls are less undulate, but stomata and hairs are abundant; of these the former are all of the same size, and surrounded by four to seven ordinary epidermis cells; the hairs are of the same types as observed upon the stem.

Viewed in transverse sections the cuticle is thick and smooth on both faces of the leaf blade, and the outer cell wall of epidermis is thickened. Large oil drops abound in the ventral epidermis. The mesophyll consists of a typical palisade tissue of one stratum, covering a very open pneumatic tissue of three to five layers. Numerous large cells containing aggregated crystals are scattered in the palisade tissue, while single rhombic crystals abound in the pneumatic tissue, especially close to the veins.

The midrib of the leaflet has a very thick-walled epidermis, and a few hypodermal strata of collenchyma on both faces, bordering on a water-storage tissue with many aggregated crystals. There is no endodermis, but a closed sheath of stereome, which surrounds a stele of several collateral mestome strands, all of which turn the leptome toward the periphery, and with the hadrome bordering on a central pith. The pith is thin-walled, and contains some few crystals, aggregated as well as single, rhombic. The much thinner lateral veins are more or less imbedded in the mesophyll, and contain only one mestome strand, surrounded by a chlorophyll-bearing parenchyma sheath. The structure of the rhachis and the petiole is identical with that of the midrib, thus containing a typical stele of several mestome strands, a sheath of stereome, and a cortex of which the peripheral strata are collenchymatic.

Juglans nigra

SEEDLING

In the Juglandaceae the cotyledons are hypogeic in all the species examined, with the exception of *Pterocarya caucasica* C. A. Mey., which germinates with the cotyledons above ground. It is a marked characteristic of the Dicotyledons that the cotyledons are epigeic, and it is only in a relatively few families that they remain underground, serving only as storage organs. Subterranean cotyledons, however, are known from trees, shrubs, and herbs, terrestrial as well as aquatic, but the Nymphaeaceae is the only family in which all the species, so far as known, germinate with the cotyledons underground and inclosed within the seed. In the other families subterranean cotyledons are characteristic of some certain groups, for instance, Vicieae, or genera: *Phryma*, *Sanguinaria*, *Caulophyllum*, *Panax*, *Melittis*, *Collinsonia*, *Quercus*, *Castanea*, *Aesculus*, *Sassafras*, *Citrus*, *Aegle*, *Mangifera*, *Persea*, *Prunus*, etc. While in some genera the majority of the species germinate with epigeic cotyledons, some exceptions occur, for instance in *Anemone*, *Oxalis*, *Clematis*, *Aristolochia*, *Phaseolus*, *Rhamnus*, etc., where some few species have the cotyledons constantly subterranean.

Characteristic of the seedlings with hypogeic cotyledons is the generally strong development of the primary root. In the Nymphaeaceae, *Nuphar*, *Nymphaea*, and *Victoria*, however, the primary

root increases but little in length during the first stages of germination, its function becoming performed by a whorl of very long root hairs developing from the base of the root as soon as the seed germinates. In *Nelumbium*, on the other hand, the root remains rudimentary, and no whorl of hairs becomes formed.[6]

The structure of the seedling of *Juglans nigra* (text fig. 1) agrees with that of *J. regia* L. as described by SCHACHT[7] and KLEBS.[8] The primary root (*R*) is stout and quite long, but it is not fusiform as in *Carya*. There is no hypocotyl, and the cotyledons remain underground, inclosed, or partly so, by the bony endocarp. They are short petioled, auriculate at base, two-lobed, and the lobes bifid. The petioles form a sheath (*S*) around the plumule, which during the first season develops into a glabrous short shoot. The first four or five leaves are very small, scalelike, and entire; the

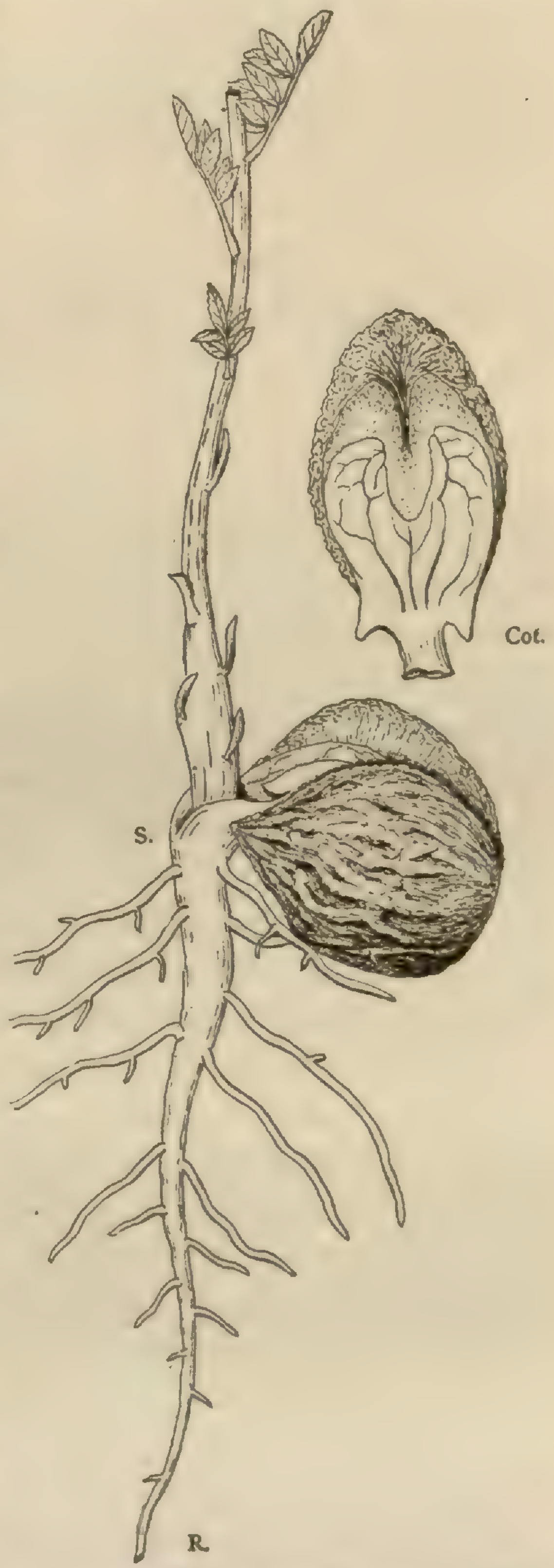


FIG. 1.—Young seedling of *J. nigra*, showing primary root (*R*), sheath formed by cotyledons (*S*), and aerial shoot; two-thirds natural size.

[6] POITEAU, Mémoire sur l'embryon des Graminées, des Cypéracées, et du *Nelumbo*. Ann. Mus. Hist. Nat. 13:397. 1809.

MIRBEL, B., Observations anatomiques et physiologiques sur le *Nelumbo nucifera*. *Ibid.* p. 474.

[7] SCHACHT, H., Beiträge zur Anatomie und Physiologie der Gewächse. p. 105. 1854.

[8] KLEBS, G., Beiträge zur Morphologie und Biologie der Keimung. Untersuch. Bot. Inst. Tübingen 1:556. 1881–1885.

succeeding are small, odd-pinnate, with three to seven leaflets. Buds are present in the axils of all the leaves, including the cotyledons; and in specimens which were injured at the apex, several of these buds had grown out into erect shoots (fig. 1).

In *Carya alba* and *C. glabra* (Mill.) Spach the seedlings agree with those of *Juglans*, but the root is fusiform. Moreover, the first two or three leaves succeeding the scalelike are unifoliate to trifoliolate, with the terminal leaflet very large, roundish, and far surpassing the lateral in size.

Root

The primary root of the seedling is stout and fleshy at the base, owing to the large development of the parenchymatic tissues, primary as well as secondary. The successive development of the various tissues may readily be seen in the same root, when examined from base to apex. In beginning with the basal swollen portion, the structure is as follows. Only some few, more or less broken strata of the primary cortex and part of the endodermis still adhere to the root, which is now covered by four or five layers of thin-walled cork of pericambial origin. Inside the cork is a broad parenchyma, the secondary cortex, rich in starch, and interrupted by two concentric bands of isolated strands of stereome. The stele shows an almost continuous zone of leptome and cambium, while the hadrome corresponds with eight distinct mestome strands. On the inner flank of the interfascicular cambium some few young vessels are visible; moreover, there are four rays of narrow protohadrome vessels readily distinguishable from the secondary by their narrow lumen. The central portion of the stele is occupied by a broad starch-bearing pith. In comparing this structure with that of the younger apical part of the same root, the following distinctions are noticeable. There is a glabrous epidermis, destitute of root hairs, and the primary cortex is a broad parenchyma without starch or crystals. Inside the endodermis is a pericambium of a single layer, in which tangential divisions have commenced, indicating the beginning formation of the cork (fig. 15, *Co*). Bordering on the pericambium is a zone of about eight layers of thin-walled parenchyma (fig. 15, C^+), representing a secondary cortex.

This tissue does not contain starch or crystals, but is interrupted, here and there, by narrow strands of secondary leptome, covered by young thin-walled stereome (fig. 16, *St*), in two concentric bands. Then follows a continuous zone of cambium connecting the four collateral mestome strands, and from which (the cambium) some few young, wide, porous vessels have become developed. Beside this secondary mestome the protohadrome vessels are very distinct, forming four short narrow rays of annular and spiral vessels.

The very commencement of the formation of these secondary tissues, the cortex and the collateral mestome strands, but not the cork, can only be traced at the youngest, the apical, portion of this root. The earliest appearance of the secondary formations depends upon a double meristem arising along the inner flank of the primary leptome, and from which secondary leptome and hadrome become formed. Outside the protohadrome the pericambium then commences to divide, forming another meristem, which in *Juglans* gives rise first to parenchyma, a secondary cortex, and a little later to a peripheral cork. Regarding the stereome, so amply represented in the secondary cortex, this tissue is totally absent from the primary structure of this root. It arises outside the leptome (fig. 16, *St*), and is formed by the secondary cortex, soon developing to distinct separate strands, arranged in one or several more or less concentric bands.

In old, thick, lateral roots the epidermis and the primary cortex are replaced by many layers of thin-walled, homogeneous cork, which surround a broad zone of compact thin-walled parenchyma (secondary cortex), the cells of which contain much starch and numerous aggregated crystals of calcium oxalate. In this secondary cortex are five or six concentric bands of isolated stereome strands (fig. 17, *St*). Viewed in longitudinal sections these stereome strands traverse the parenchyma in wavy, not parallel lines. The stele contains a peripheral zone of almost continuous leptome, also several strata of cambium, beside a dense mass of hadrome, in which wide porous tracheids with bordered pits are quite conspicuous. Thick-walled libriform, and thin-walled parenchyma with starch represent also a large part of the stele. The medullary

rays (fig. 17, *PR*) are narrow, mostly of a single row of cells, compressed radially, and filled with starch. The protohadrome vessels are readily seen in the center of the root, surrounded by strata of thick-walled conjunctive tissue; no pith is developed.

STEM

The young shoot, examined in the early spring, is densely covered with hairs, especially glandular. Unicellular, pointed hairs are also common, and these occur in clusters of from two to fifteen, or even more. The cuticle is thick, smooth, and the epidermis is thick-walled. During the fall the epidermis is replaced by a hypodermal cork of heterogeneous structure, thin-walled strata alternating with thick-walled. This cork is developed from the hypodermal stratum of a collenchyma. Inside the collenchyma is a broad, compact, thin-walled parenchyma, filled with starch and large aggregated crystals. Two concentric bands of stereome are developed in the inner part of the cortex. There is no endodermis, and the stele shows a continuous zone of leptome, interspersed with cells containing single rhombic crystals. The cambium is well represented, and in the hadrome the porous vessels are remarkably thick-walled. Cells containing single crystals occur also in the hadrome. The medullary rays are narrow, mostly of a single row of cells, containing starch. There is a relatively thick-walled pith, porous, filled with starch and aggregated crystals, and becoming soon septate as in *Juglans regia* and *Pterocarya*, as mentioned by SOLEREDER. A corresponding structure is exhibited by the old thick branches, but in these the stereome occurs in a larger number of concentric bands, twelve or even more. The pith also is here divided by transverse septa.

Finally may be mentioned that the internodes of the young seedling are perfectly glabrous, and a cork is developed from the hypodermal layer of the cortex, or from the stratum inside this; both cases may be observed in the same section. There is no collenchyma in these internodes during the first season, and the cortex is thin-walled throughout, destitute of starch and crystals. Inside the barely distinguishable endodermis are four or five layers of thick-walled stereome, forming arches, more or less continuous

as a closed sheath. Bordering on the stereome is a broad zone of thin-walled parenchyma, with narrow isolated strands of leptome. The cambium forms a closed ring, and the hadrome is in deep rays with much thick-walled libriform. The pith is homogeneous, thin-walled, filled with starch, but solid, not septate as in the shoots of the mature tree.

Leaf

When unfolding, the leaves are very hairy, especially on the dorsal face, and the hairs are of the types that occur on the young shoots. The stomata are confined to the dorsal face, and lack subsidiary cells. They represent two sizes, both of which are equally common. Viewed in superficial sections the lateral walls of epidermis are straight on both faces of the leaf blade. With regard to the distribution of the various hairs, the pointed, fasciculate, abound beneath the veins, and are absent from the ventral face; the glandular are common on both faces; but the largest type, sessile with a large head, are confined to above and below the mesophyll. The mesophyll consists of a compact palisade tissue of a single stratum, or sometimes two strata (fig. 18, *P*), covering a very open pneumatic tissue with numerous large cells containing aggregated crystals, especially close to the epidermis.

The midrib is supported by several hypodermal layers of collenchyma on both faces, and is furthermore surrounded by a water-storage tissue. There is no endodermis, but a closed sheath of thick-walled stereome in several strata surrounding the steloid midvein, which is composed of an obtusely triangular band (in cross-sections) of collateral mestome strands inclosing a central parenchyma, a pith. In these mestome strands the hadrome faces the pith, while the leptome turns toward the periphery, even in the ventral part of the stele. Characteristic of the hadrome is the abundance of thin-walled parenchyma in continuation with the vessels. The lateral veins contain only single mestome strands which are supported by stereome extending to the ventral and dorsal epidermis, broken on the sides by thin-walled cells of a parenchyma sheath.

Between the leaflets the rhachilla is hemicylindric (in cross-sections), very hairy, with long stalked glandular hairs. Several

hypodermal and continuous layers of collenchyma surround a broad thin-walled cortex, rich in chlorophyll, and with some aggregated crystals. No endodermis is developed, but a closed sheath of stereome surrounds a stele of collateral mestome strands as in the midrib of the blade. The pith is solid, not divided into septa.

Examined just below the basal pair of leaflets, the petiole is hairy like the rhachilla, and shows the same structure, except that there are two thin collateral mestome strands located in the cortex, thus outside the stele, and in these the leptome is covered by a few layers of stereome; the pith is solid.

Cotyledons

Although completely subterranean, the epidermis of the cotyledons shows stomata, but relatively only a few, on both faces of the thick fleshy blade. The lateral cell walls are straight on both faces, and the lumen is about the same, or slightly wider on the ventral face. The mesophyll lacks palisade cells, and is composed of a large, thin-walled, compact parenchyma of roundish cells. All the mestome strands are single, collateral, surrounded by parenchyma sheaths, and are imbedded in the mesophyll. The leptome is generally much better represented than the hadrome, and no mechanical tissues are developed in these leaves.

Juglans cinerea shows the same structure as *J. nigra*, with the only exception that the pericycle in the stem represents an almost closed sheath interspersed with large, thick-walled, and porous sclereids. The pith is discoid, and the diaphragms contain many aggregated crystals. The pointed hairs of the leaf are more abundant than in *J. nigra*, and occur mostly in clusters of two to eight on the dorsal face of the blade.

Characteristic of *Juglans* and *Carya* is thus the ample representation of mechanical tissues, as collenchyma, stereome, and liriform. Of these the collenchyma occurs in the stem, the periphery of the cortex proper, and in the leaves as hypodermal strata on both faces of the midrib. The stereome occurs as a secondary tissue in the cortex of the root and stem, as well as pericyclic arches or, sometimes, forming a closed sheath, interspersed with sclereids

in *J. cinerea;* it occurs also in the leaves forming a sheath around the midrib. Thick-walled libriform is noticeable already in the apical internodes of the seedling, and in branches of the mature tree the hadrome is divided by broad tangential bands of this tissue. In old roots of *Juglans* the libriform is much in evidence.

With respect to the distribution of the calcium-oxalate as single or aggregated crystals, SOLEREDER (Anatomie Dicot.) calls attention to the very varied occurrence of these types of crystals. In *Juglans nigra* aggregated crystals were observed in the inner part of the cortex and pith of the stem, as well as in the pneumatic tissue of the leaf. On the other hand, single crystals were noticed in the leptome and hadrome of the stem. In *Carya alba* aggregated crystals were observed in the cortex and leptome of the stem, as well as in the palisade tissue of the leaf, and in the pith of the steloid midrib. Single crystals, on the other hand, were found in the collenchyma of the stem, as well as in the pneumatic tissue of the leaf and in the pith of the steloid midrib; thus both types of crystals occur in the pith of the midrib.

Of greater interest, however, is the singular structure of the pith in *Juglans* and *Pterocarya*. The history of this structure, the discoid pith, dates back to GREW,[9] who discovered it in *Juglans*. By MIRBEL[10] it was mentioned as peculiar to *Phytolacca, Nyssa,* and *Juglans*. DE CANDOLLE[11] found a discoid pith in *Jasminum officinale*. MORREN,[12] in describing discoid piths of plants, enumerates several other plants, for instance, *Begonia argyrostigma*, while this writer found the pith to be solid in *B. undulata, B. semperflorens,* and *B. papillosa*. According to SOLEREDER the discoid pith is characteristic of two herbs, *Diplotaxis* and *Pedalium*, and among woody plants he enumerates *Wormia* (Dilleniaceae), *Fouquiera* (Tamariscineae), *Prinsepia* (Chrysobalanaceae), *Aucuba, Halesia, Paulownia, Daphniphyllum* (Daphniphyllaceae), as well as the

[9] GREW, N., Anatome plantarum. *pl. 19. fig. 4.* 1682.

[10] MIRBEL, B., Elémens de Physiologie végétale et de Botanique. 1:112. 1815.

[11] DE CANDOLLE, A. P., Organographie. 1:167. 1827.

[12] MORREN, C., On the discoid piths of plants. Ann. Nat. Hist. London. 4:73. 1839–1840.

genera mentioned in the preceding. By FOXWORTHY[13] a general discussion of discoid pith has been presented. Finally by the writer[14] the structure of the pith in *Phytolacca decandra* L. has been described and figured.

While the discoid pith is thus characteristic of the species of certain genera, it has been shown that in *Begonia*, *Forsythia*, *Jasminum*, and *Phytolacca* this structure occurs only in certain species. *Juglans* and *Pterocarya* are definitely separated from the other genera by the possession of a discoid pith. It is a very interesting structure, which, however, must not be confounded with cases where the pith is solid, and divided by horizontal diaphragms of sclerotic cells, so characteristic of many Magnoliaceae, Anonaceae, Ternstroemiaceae, and Convolvulaceae.

CLINTON, MD.

EXPLANATION OF PLATES XV, XVI

PLATE XV

Carya alba

Figs. 2, 3, 4, 7, 8, 10, 11, 13, and 14 are enlarged.

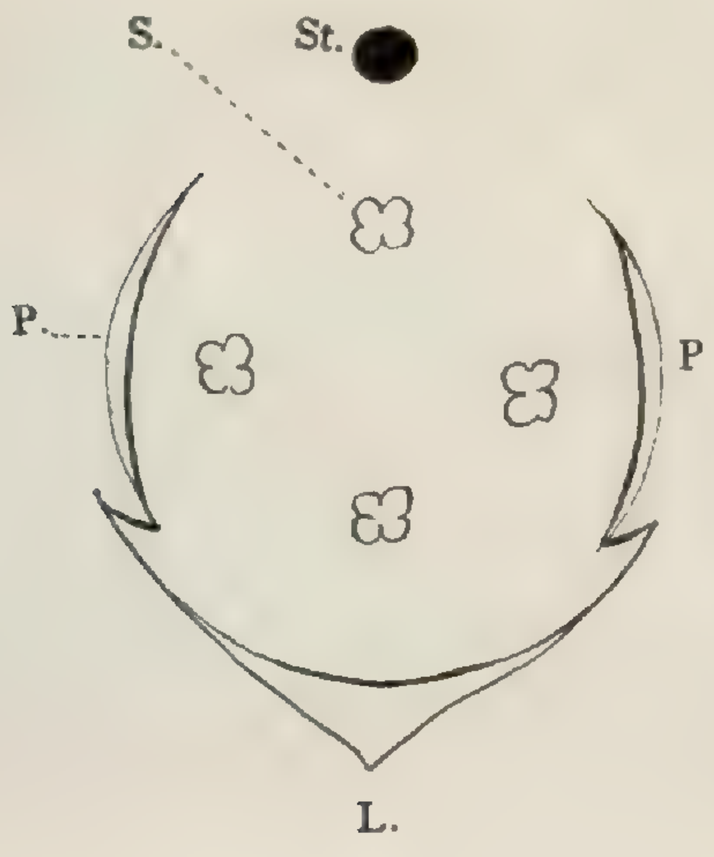
FIG. 1.—Staminate flower: *St*, stem; *L*, bract; *P*, prophylla; *S*, stamens.
FIG. 2.—Involucre of staminate flower, seen from outside.
FIG. 3.—Staminate flower, side view.
FIG. 4.—Stamen.
FIG. 5.—Pistillate flower: *PL*, perianth leaves; other letters as preceding.
FIG. 6.—Pistillate flower with single perianth leaf.
FIG. 7.—Pistillate flower, side view; *PS*, petiole.
FIG. 8.—Pistillate flower, seen from above.
FIG. 9.—Cross-section of inner part of primary root of seedling in second year: *St*, stereome strands outside secondary leptome (*L*); *Camb*, cambium; *H*, hadrome; *PR*, parenchymatic ray; *P*, pith; × 320.

Juglans nigra

FIG. 10.—Staminate flower, seen from outside; stamens removed.
FIG. 11.—Two stamens, side and front view.
FIG. 12.—Branch with pistillate flowers; natural size.
FIG. 13.—Longitudinal section of pistillate flower; *P*+, perianth leaves.
FIG. 14.—Cross-section of stigma.

[13] FOXWORTHY, E. W., Discoid pith in woody plants. Proc. Indiana Acad. Sci. p. 191. 1903.

[14] HOLM, THEO., Medicinal plants of North America. 9. *Phytolacca decandra* L. Merck's Report. p. 312. 1907.



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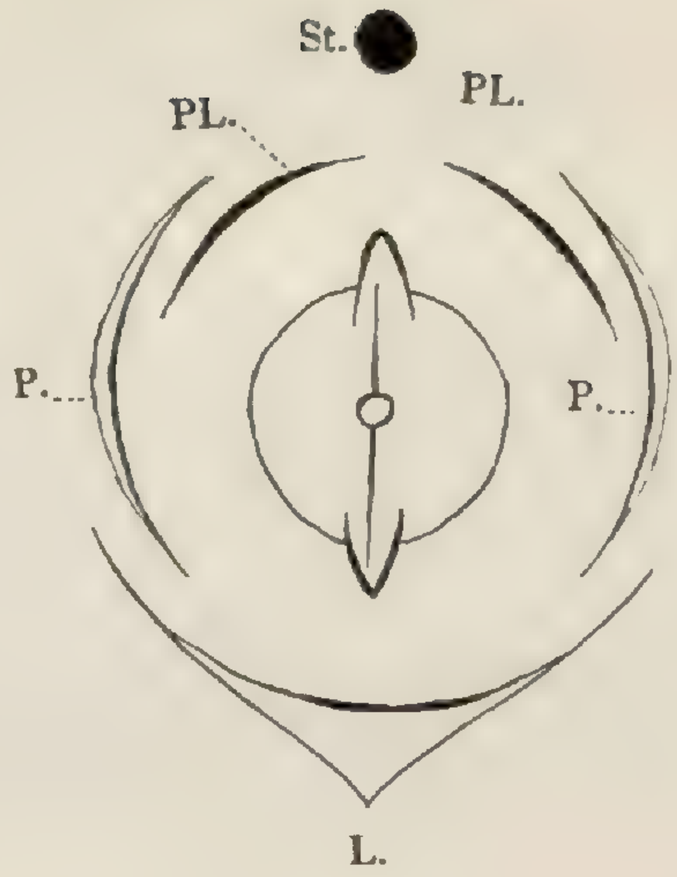

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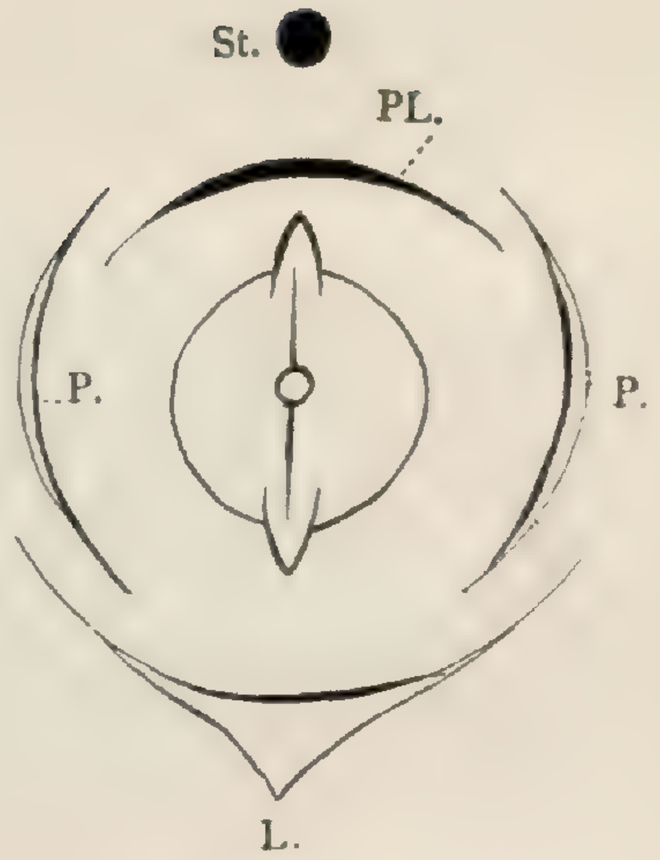

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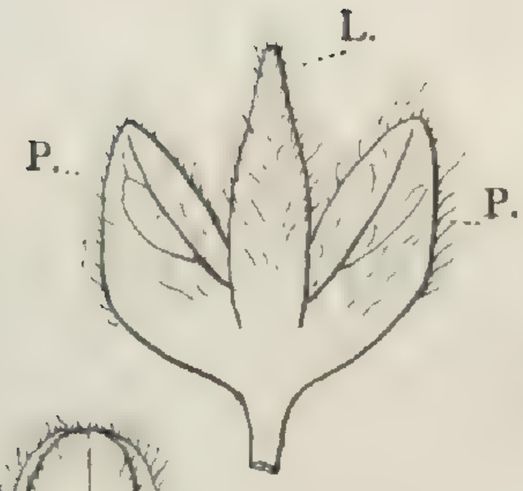

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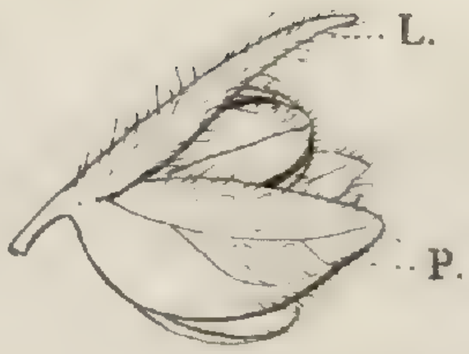

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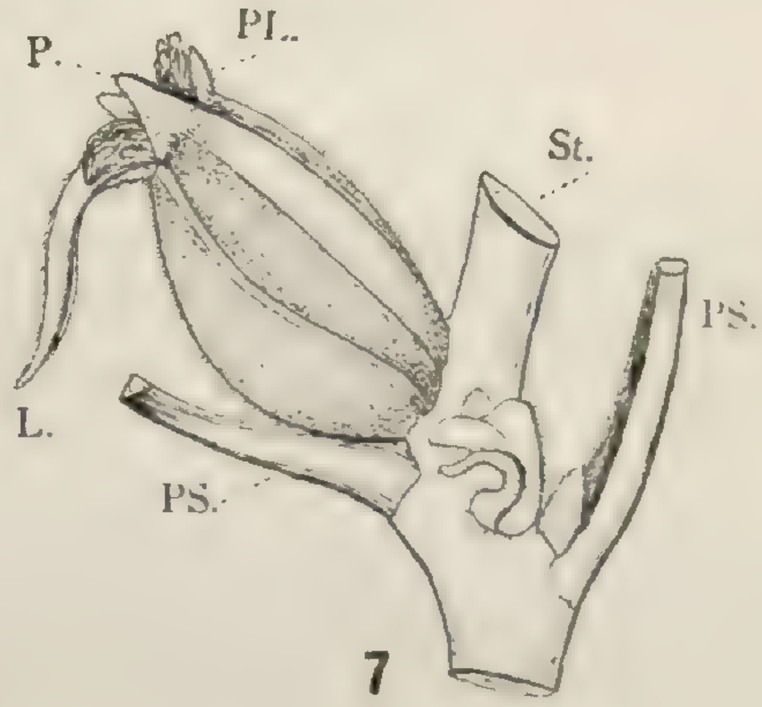

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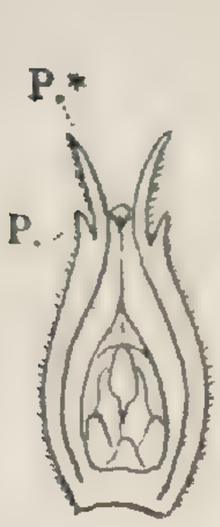

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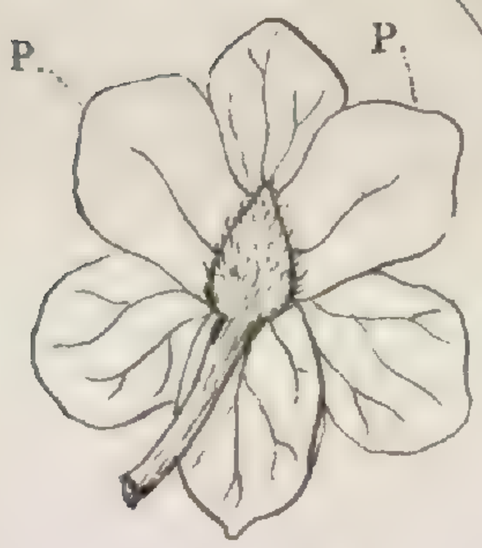

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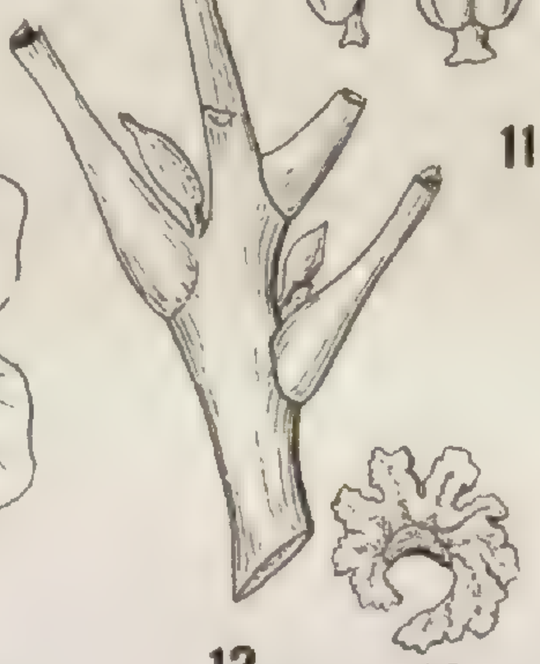

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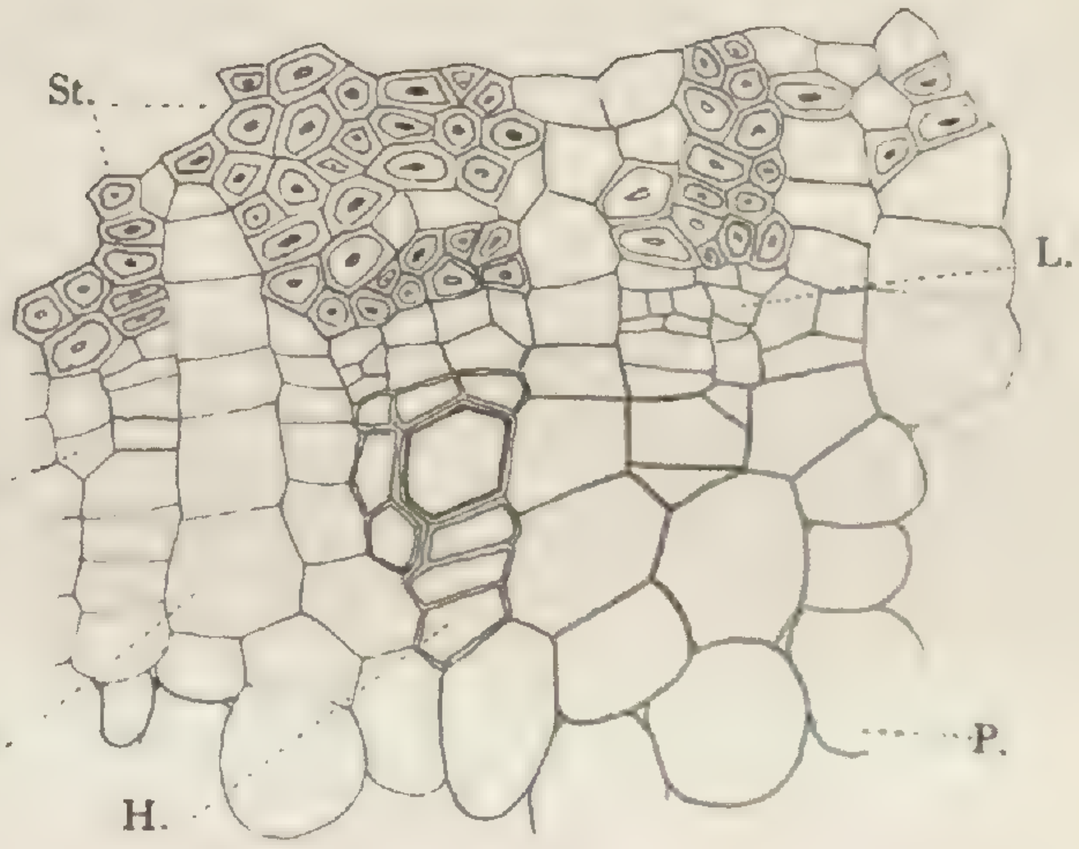

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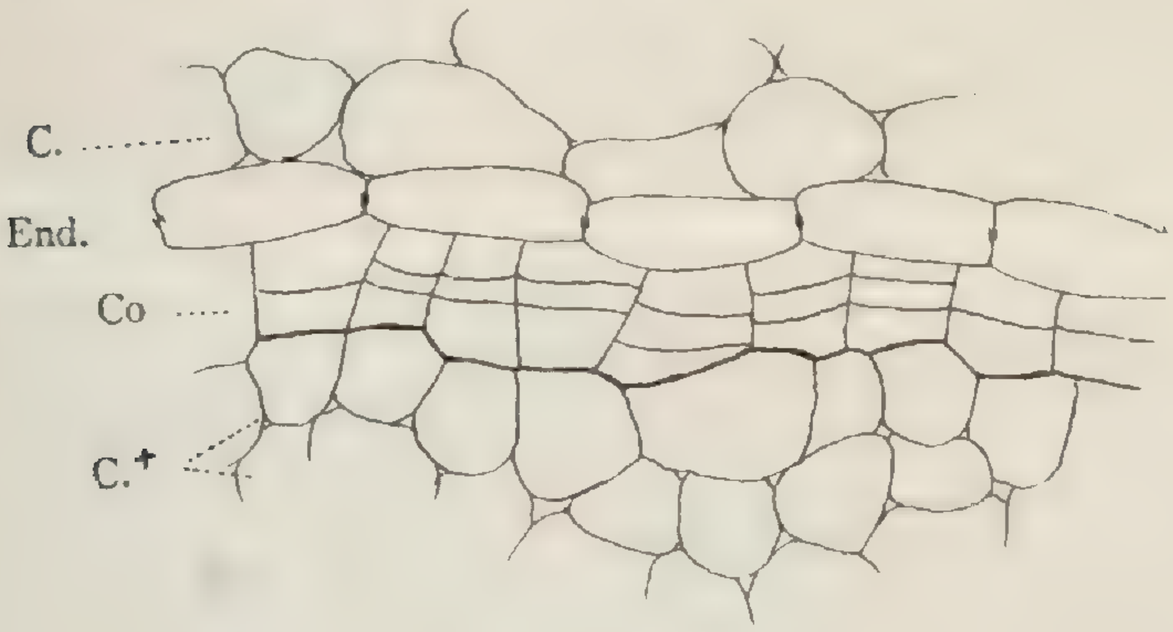
HOLM on CARYA and JUGLANS



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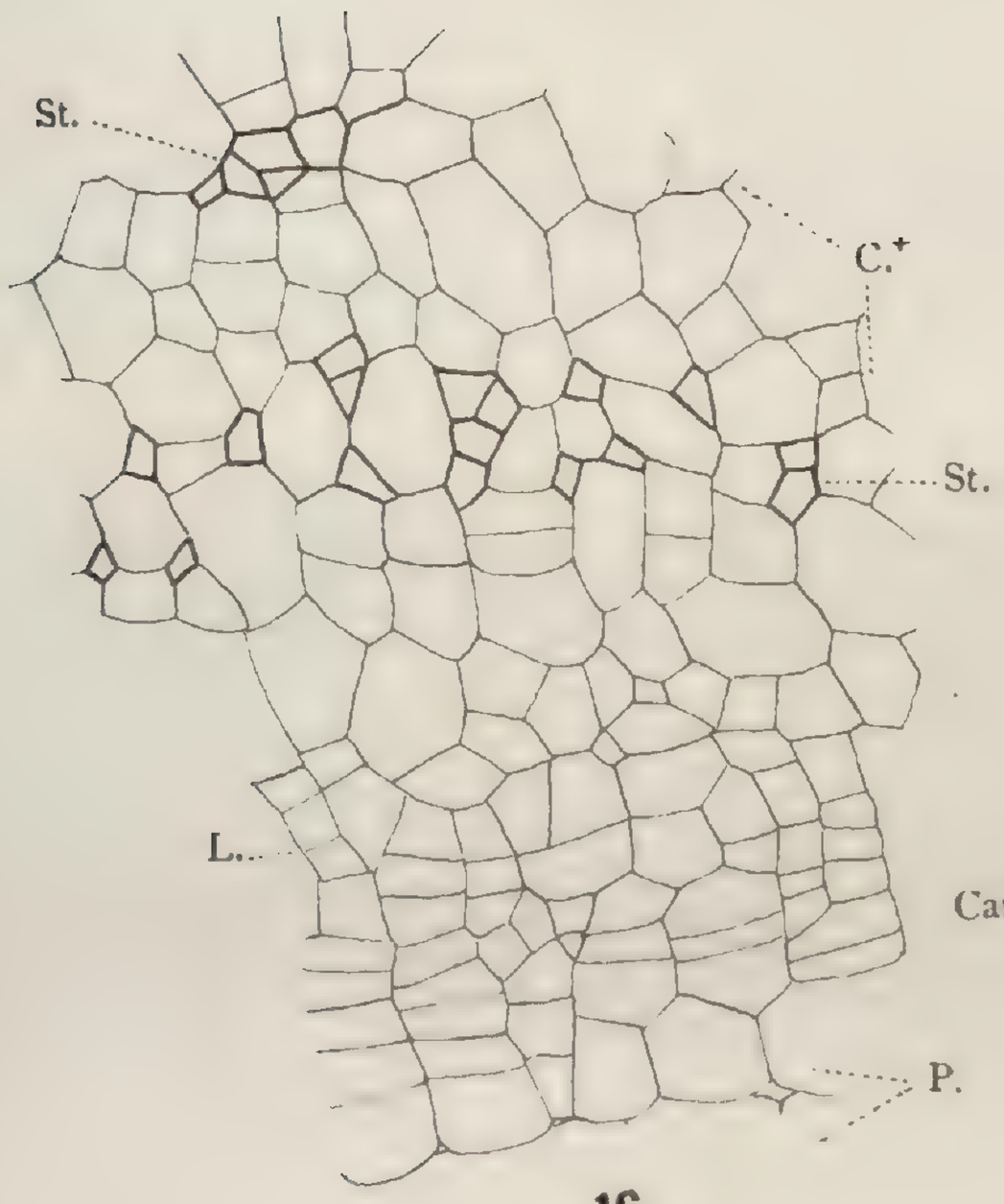

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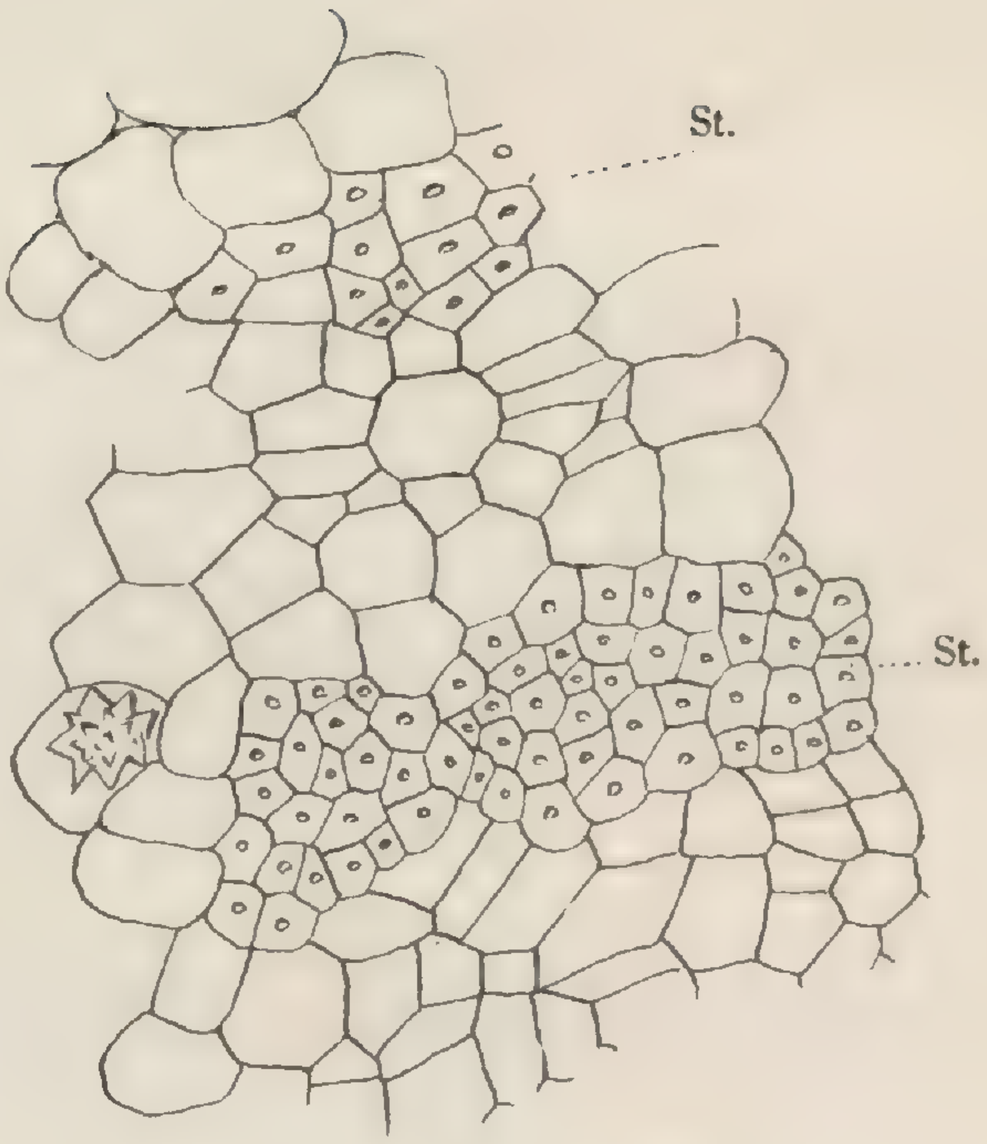

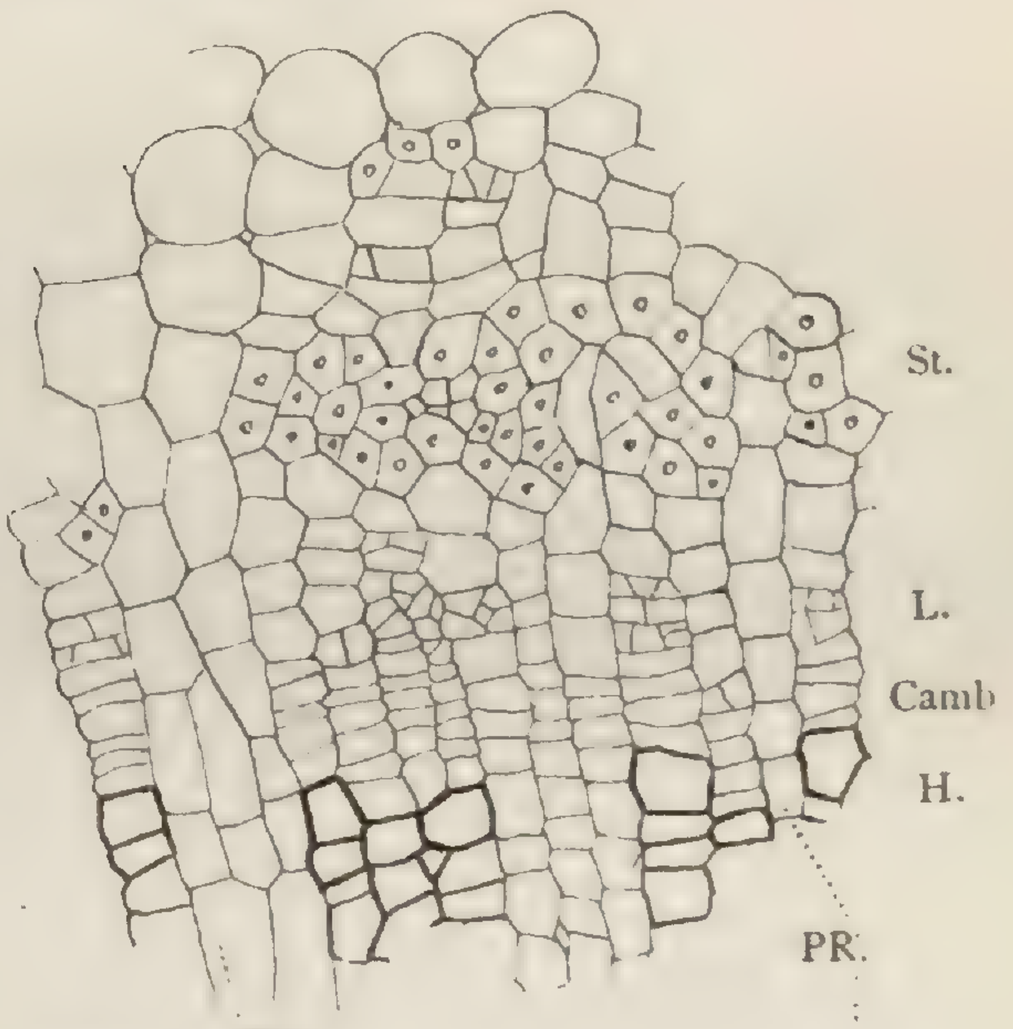

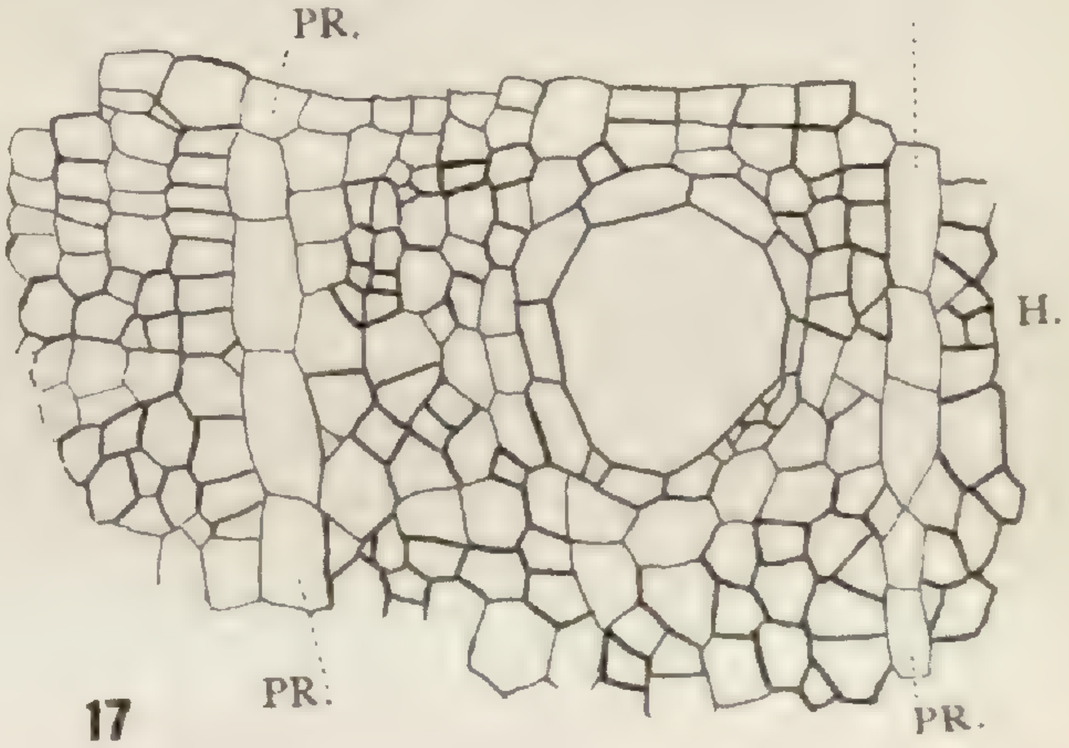

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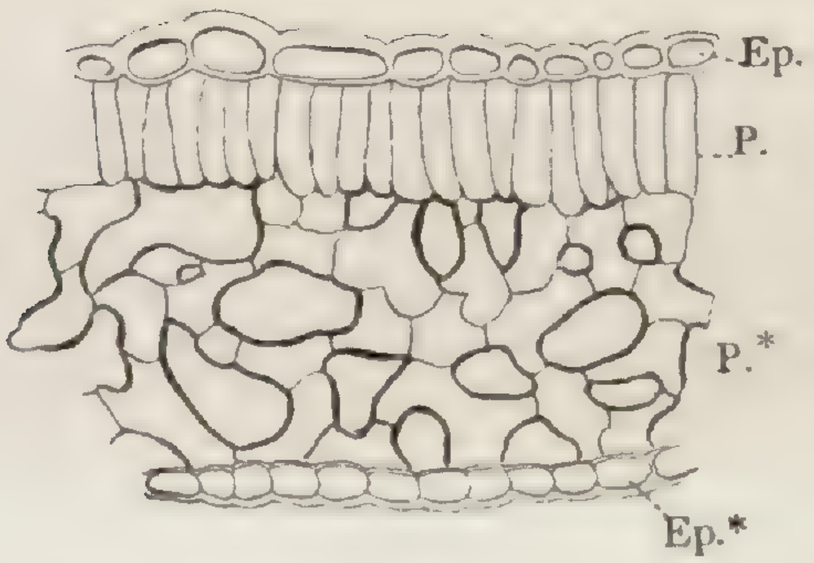

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HOLM on CARYA and JUGLANS

PLATE XVI

Juglans nigra

FIG. 15.—Cross-section of primary root of young seedling a month old: *C*, inner part of primary cortex; *End*, endodermis; *Co*, pericambial cork; *C*+, peripheral part of secondary cortex; × 320.

FIG. 16.—Inner part of same root (fig. 15): *C*+, secondary cortex with strands of stereome (*St*); *L*, leptome; *Camb*, cambium; *P*, outermost layer of pith; ×320.

FIG. 17.—Three cross-sections of old lateral root; *PR*, parenchymatic ray; ×320.

FIG. 18.—Cross-section of leaf: *Ep*, ventral, *Ep*+, dorsal epidermis; *P*, palisade tissue; *P*+, pneumatic tissue; ×320.

PHYLOGENETIC POSITION OF THE BACTERIA[1]

HILDA HEMPL HELLER

The subject of the phylogenetic position of the bacteria has been approached by many students. Early workers came to no more diverse conclusions than do modern ones. Some investigators, for example NÄGELI (**24**) and GÖTSCHLICH (**14**), have placed the bacteria with the fungi, while COHN (**9**), MIGULA (**22**), and SACHS (**26**) placed them with the algae. The early workers who assigned the bacteria to the fungi did so because both fungi and bacteria lack chlorophyll, and may thus be regarded as similarly degenerate algae, and because there are genera such as *Corynebacterium*, *Actinomyces*, *Streptothrix*, and *Oidium*, that may well be regarded as transitional forms. Classifiers of the fungi have not sufficiently emphasized the fact that in a group where chlorophyll is absent there is no compelling reason for presuming that the simpler forms, the bacteria, were descended from the higher ones, as the workers thought who considered them as directly descended from the algae. Even DE BARY (**1**), although he uses NÄGELI'S name "Schizomycetes" (fission fungi) for the bacteria, insists that they are not fungi, nor closely related to or descended from fungi.

The reason for classing the bacteria as a subordinate group of the algae has usually been the exceedingly close morphological resemblance of the higher bacteria to the blue-green algae (Cyanophyceae or Myxophyceae). COHN was the first to emphasize the relationship between these groups. The Cyanophyceae were long thought to be the most simple autotrophic forms. More modern systematists have separated the blue-green algae from those with sexual reproduction, and have united them with the bacteria. Thus ENGLER (**12**), in his second phylum Schizophyta, included only the two classes Schizomycetes and Schizophyceae; WARMING (**28**) made a similar division of his class Schizophyta; while BESSEY (**3**) in his phylum Myxophyceae, class Archiplastideae

[1] From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.

(blue-green algae without nuclear membrane), placed the Bacteriales as an order coordinate with two orders of the blue-green algae. BÜTSCHLI (6) and KLEBS (19) emphasized the common characters of the bacteria and the protozoa.

Today the question is apparently no nearer a solution than it was forty years ago. The various views are based on the consideration of different life manifestations. Close relationship between bacteria and protozoa, however, is no longer emphasized. The principal views held today are three: (1) that the bacteria are members of the group of fungi, (2) that they are derived from or closely related to the Cyanophyceae, and (3) that they are the primitive forms from which fungi and algae are derived. The first two opinions are held by morphologists. Those who have regarded the manner of division and sporulation, the external characters of the organism so to speak, and those who hold that bacteria possess a small oval or round nucleus, have allied the bacteria with the fungi. Others who have studied their nuclear structure have allied them with the Cyanophyceae. Chemists and general students of evolution have recently considered them as ancestors of the other groups.

The morphological field has been reviewed carefully by MEYER (21), who holds that the closest affinities of the bacteria are with the fungi. The bacterial nucleus, according to his conception, is similar to the nucleus of the fungi. SWELLENGREBEL (27), GARDNER (13), and DOBELL (11) have observed structures, which they believe to be nuclei, that have marked resemblance to the chromophyll portion of the central bodies of the Cyanophyceae. GUILLIERMOND (15) and a number of other workers hold the bacterial nucleus to be "chromidial" or finely distributed in the cytoplasm. DOBELL finds structures resembling all these types, which he holds to be nuclei, and he believes the bacteria to be related most closely to the Cyanophyceae. WEST (29) described a blue-green alga, *Myxobactron*, which shows no differentiation of its protoplasm. PARAVICINI (25) has recently described minute compact structures that he believes to be nuclei.

JENSEN (18) rearranged the bacteria on a chemical basis, and defined their relation to the algae, fungi, and protozoa, presuming

that the earth was dark when life began, and that chlorophyll-free bacteria, probably those capable of oxidizing methane, were the earliest forms of life with which we are familiar today. JENSEN derived the blue-green algae from the sulphur bacteria, the fungi from the oxidizing bacteria by way of the Actinomycetes, and the higher bacteria from the earliest nitrogen-reducing organisms. KLIGLER (**20**) was also of the opinion that bacteria may well have been the earliest forms of life, and he placed the methane-oxidizing type at the base of his tree. BREED, CONN, and BAKER (**4**) pointed out that there is no proof that the world was dark when life began; that in case it was light the ancestors of the blue-green algae or of the phototrophic pigment bacteria, which use sunlight to metabolize organic substances, may have been the most primitive forms; or that the most primitive form may be entirely unknown to us. Thus we see that because of the discovery of the existence of autotrophic bacteria the old question of the origin of the bacterial group is again open.

When the synthesis of inorganic substances into organic material was thought to be possible only by the aid of chlorophyll, the natural trend of evolutionary reasoning led to the derivation of other forms of life from simple chlorophyll-containing ones. Bacteria apparently are simpler than the most simple chlorophyll-bearing algae. They were therefore thought to be degenerate. Workers who saw in them affinities with the chlorophyll-free fungi were not careful to state what their relationship with the fungi really might have been. The reader is usually left with the impression that they are in an intermediate position or related to the higher fungi. MEYER, who excluded the *Thiobacteria*, *Chlamydobacteria*, and *Myxobacteria* from his Eubacteria or bacteria proper, placed his group as the second class of the Eumycetes next to the Phycomycetes or algal fungi. CLAYPOLE (**7**) considered both bacteria and fungi to be derived from the leptothrix-tuberculosis group.

A rather suprising paper has recently appeared by BERGSTRAND (**2**), who has observed the budding and branching of *Corynebacterium* and other forms, and believes that the bacteria are closely related to the fungi. Budding and binary fission are not so different in their nature that they should be considered very important

characters. One genus of yeasts, the *Schizosaccharomyces*, divide as do the bacteria. Apparently typhoid bacilli may either bud or divide by fission HORT (16). Upon this one character of budding, BERGSTRAND lays so much emphasis that he refuses to consider other characters, also morphological, which show similarity between the bacteria and other forms: "To discuss further the eventual relationship of Cyanophyceae to bacteria does not seem necessary, because any such theory would appear false at the moment that it became clear that bacteria are more closely related to fungi, as I shall show." It must be noted that, like MEYER, BERGSTRAND excludes from his bacterial group the higher bacteria which do not resemble the fungi as much as they do the algae. One would be equally justified in naming as bacteria all the chlorophyll-free rods except the branching and budding ones. BERGSTRAND defines the bacteria as Fungi Imperfecti. The Fungi Imperfecti are an entirely artificial group comprising fungi that have not developed sexual characters, those that have lost such characters, and those that have not been studied sufficiently to determine their true relationships. BERGSTRAND concludes that bacteria are to be regarded as Fungi Imperfecti that have developed through the reduction of higher forms, and not as lowly primordial organisms to be placed at the very beginning of the organic world. An example of his logic is as follows: "Of course if one regards bacteria as Fungi Imperfecti one cannot accept the theory that the chromatin is spread diffusely in the cell body, because this assumes it would seem a much lower developmental stage."

It is not the intention of this paper to criticize workers for connecting bacteria with fungi because of morphologic relationships between the two groups. BERGSTRAND'S observations serve to strengthen the tie between the fungi and the bacteria, but the lightness with which he proposes the degeneracy of the latter forms from the former is a novel process to comparative biological reasoning. The trend of evolution is rarely in the direction of degeneracy. Degeneracy occurs as a consequence of a parasitic habit or because of abundant food supply. It is usually accompanied by vestigial traces of a former complexity. The characters which the bacteria and fungi have in common are not manifestly vestigial in the

bacteria. The supposed loss of sexual characters among the fungi has been attributed to their change from water forms to air forms, but bacteria are not air forms. The theory of the degeneration of the bacteria from the algae was a very peculiar one, imposed by ignorance of certain primitive bacteria. It is now known that bacteria exist which are autotrophic and can secure growth energy from inorganic carbon, so that their lack of chlorophyll is no longer a reason for considering them degenerated from the chlorophyll-containing forms. The existence of autotrophic fungi, to my knowledge, has never been demonstrated.

There is a simple group, therefore, the members of which are autotrophic; and two diverse complex groups, one of which (the fungi) is not autotrophic and may not be homogeneous. Both of these complex groups show marked resemblances to the simple one. JENSEN'S scheme, which derives both of them from the simple one, is not to be lightly thrown aside. It coincides too well with the general scheme of evolution. We may, if we wish, consider the question entirely open, but nomenclature and classification should be so formulated that they do not deliberately mislead the amateur on the subject of these relationships. Formerly the tendency in botanical classification was to make a treelike structure, throwing groups together that had but superficial resemblances, but classifiers today are more prone to refuse to indicate relationships where descent is not fairly certain, and to group the plants in phyla like the zoological phyla, whose connections may or may not be understood.

The bacteria, fungi, and blue-green algae, therefore, may be all in one phylum, or may be placed in three separate phyla, but to place the bacteria with either fungi or Cyanophyceae is inconsistent, because it leaves out of consideration the third group which may be equally related to the bacteria. Probably the trend of classification would favor the separation of these groups into three separate phyla, for to place the fungi and Cyanophyceae together is rather stretching the limits of the botanist's conception of a phylum. Moreover, in view of the existing divergent opinions, a classification that does not commit one on the subject of these relationships is preferable. A name for the phylum that is to

contain the bacteria only should not indicate for them a subordinate position in another group as does the name "Schizomycetes," proposed by NÄGELI (**23**) in 1857 for a mongrel group which contained bacteria, sporozoa, and oscillaria, a group whose affinities he hesitated to suggest. The connotation of this term has always been "fission fungi," and its German form "Spaltpilze" has been widely used. And yet BUCHANAN (**5**) finds it entirely appropriate and valid and proceeds to place his Schizomycetes with the Cyanophyceae. Article 51, division 4, of the Vienna rules (**17**) considers the name Schizomycetes as invalid. "Everyone should refuse to admit a name . . . . when the group which it designates embraces elements altogether incoherent, or when it becomes a permanent source of confusion and error."

We should choose for the bacterial phylum a name that will immediately be understood by the non-professional worker. Names like *Phytozoidia* Perty of course are objectionable. *Vibrio* Ehrenberg probably included certain infusoria as well as bacteria. *Vibrionia* Cohn did not include forms later studied by that author. *Bacteria* Cohn (**8**) probably included all the forms that we today call bacteria except *Beggiatoa*, and it did not include members of other groups. As the Committee of the Society of American Bacteriologists (**10**) places 1880 as the date at which considerations of priority are to commence, we are free to choose from among these names. Bacteria implies no relationship to other groups. It is otherwise highly suitable because it is understood by laymen and is short and euphonious. The following was COHN'S conception of the group: "Die Bacterien sind chlorophyllose Zellen von kugeliger, oblonger oder cylindrischer, mitunter gedrehter oder gekrümmter Gestalt, welche ausschliesslich durch Querteilung sich vermehren, und entweder isoliert oder in Zellfamilien vegetieren."

In consideration of the fact that no relationship of the bacteria to other groups has been generally accepted the following phylum is proposed:

BACTERIA (nov. phyl.).—Simple one-celled plants that multiply typically by binary fission and occasionally by budding. They show no form of sexual multiplication. They rarely contain cellulose and do not contain chlorophyll or phycocyanin.

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ODONTOPTERIS GENUINA IN RHODE ISLAND

EDA M. ROUND

(WITH FIVE FIGURES)

One of the most characteristic and common fossils of Rhode Island is *Odontopteris genuina* Grand'Eury. These plants apparently grew to great size around the coal swamps of the Narragansett Basin during the Carboniferous, somewhat like the tree ferns of the tropical forests of the present day (fig. 1). The fronds appear to have been bifurcate, the angle formed by the branches being about 90° (fig. 2). The rachis is striated and clothed with short pinnae, the latter having enlarged pinnules at their tips and being more separated than those of the expanded parts of the frond. The pinnae vary considerably, sometimes being short or at other times attaining a length of over 15 cm. The pinnules often vary in shape on the same specimen, some being falcate and acute, while others are oval and rounded at their apices. The acute type of *O. genuina* is very common in the state, and may have come about as a result of the conditions under which the fossils were originally imbedded. The pinnules appear to have been firm in texture and convex or "bombe" in shape. If these shapes were squarely imbedded they would appear oval (fig. 3*a*) when fossilized, while more pointed effects would result from preservation at a slight angle (fig. 3*b;* fig. 4*a*, *b*), and long, narrow effects from still greater angles (fig. 5*a*). While these forms have pinnules 3–8 mm. broad by 10–16 mm. long, the illustrations from Pawtucket show much larger sizes and resemble those figured by ZEILLER[1] from Commentry, France. The Pawtucket specimens do not appear to have been as firm and thick as the smaller Rhode Island types, and the borders are inclined to be less even. The pinnules also were evidently flat rather than convex in shape and somewhat cyclopterid in appearance (fig. 3*c*, *d;* fig. 5*b*).

[1] ZEILLER, C. R., Études sur le Terrain Houiller de Commentry. *pl. 24.* 1888.

It appears that *O. genuina* has frequently been listed among Rhode Island fossils under the name *O. brardii* Brgt., presumably

FIG. 1.—*Odontopteris genuina:* tip of frond (distorted); reduced ½.

owing to the numerous examples of falcate forms in evidence. A careful study of the veining upon good material, however,

reveals a much more complex system than that of *O. brardii.*[2] In general the *O. genuina* has a thin medial nerve, distinct almost to the apex, while the lateral veins spread at very acute angles

FIG. 2.—*Odontopteris genuina:* mode of branching; reduced ½.

and fork in passing to the border one to four times, the lowest or outermost only coming from the rachis. Typical *O. brardii,* on the other hand, is described as having veins all of which come

[2] BRONGNIART, A., Histoire des végétaux fossiles. *pls. 75, 76.* 1828.

FIG. 3.—*Odontopteris genuina:* slightly reduced.

FIG. 4.—*Odontopteris genuina: a*, natural size; *b*, ×2.

from the rachis, a condition which the writer has never observed in Rhode Island specimens.

FIG. 5.—*Odontopteris genuina:* natural size.

Odontopteris genuina has been found in eight localities in the state, namely, Portsmouth, Boyden Heights, The Tunnel, Provi-

dence, Pawtucket, Arlington, Cranston, and Warwick. These rocks are now in the collection of the geological department of Brown University. As they never appear waterworn it may be inferred that these plants fringed the coal marshes of the Narragansett Basin in the Carboniferous, and were buried and fossilized near their places of growth. Most of these fossiliferous materials are preserved in fine grained black shale. The specimen from Boyden Heights, however, is of sandstone, a material not generally fossiliferous in the state except as the matrix of coarse forms like Calamites (fig. 3*a*).

With such abundance of preserved material as is represented by *O. genuina* in Rhode Island, it seems significant that no fruited pinnae are in evidence. It has been proved by KIDSTON,[3] however, that many of the so-called fossil "ferns" were really Pteridosperms or Cycadofilicales. Many detached seeds are found in the rocks of Rhode Island, proof that the ancestors of modern flowering plants were denisons of the coal forests of the state, among which it seems probable that *O. genuina* may sometime be included.

[3] KIDSTON, R., Les végétaux houillers recueillis dans le Hainaut belge. Mém. Mus. Roy. Hist. Nat. Belg. 4:5. 1911.

BRIEFER ARTICLES

ROOT DEVELOPMENT OF WHEAT SEEDLINGS

(WITH ONE FIGURE)

In a study of the salt requirements of wheat in water cultures, certain conditions under which wheat seedlings developed relatively large root systems were noted. Wheat seedlings with shoots 8–10 cm. high and roots 10–12 cm. long were set out according to the usual method employed for solution culture experiments, in two quart Mason jars filled with tap water from the laboratory. The cultures were allowed to grow for six weeks at a temperature range of 22–32°C. and without renewal of the tap water. At the end of this period the tops of the cultures had grown about 12–16 cm. high (having gained from 2 to 4 cm.) and the root mass measured 70–80 cm. in length. In some cultures, however, single roots had attained a length of over 100 cm. So far as the total dry weight of these cultures was concerned, it may be stated that about one-half was contained in the roots.

It was at first thought that the relatively low total salt concentration of the tap water was responsible for the results. The tap water of the laboratory contained a total salt concentration whose osmotic value was calculated to be approximately equal to 0.1 atmosphere pressure. To test this supposition as being the cause for the extraordinary long root growth of the wheat seedlings, several different kinds of complete nutrient solutions were prepared, each having a total salt concentration giving an osmotic value equal to about 0.1 atmosphere pressure, and these were used as the culture media for wheat. These dilute solutions, which contained all of the chemical constituents essential for plant growth, proved to be relatively poor media for the root development of wheat seedlings. Another set of tests, however, with solutions of the same salts and salt proportions as those of these dilute solutions but of greater total concentration (0.5 atmosphere), proved to be very good media for the root development of wheat seedlings. These results suggested that it might be the absence or the deficiency of an element in the tap water that was responsible for the results. Tests were then made using nutrient solutions of a total salt concentration equal to give about 0.5 atmosphere osmotic pressure, but modified so as to omit one

of the elements considered essential for normal plant growth. Wheat seedlings with shoots 8–10 cm. high and roots 10–12 cm. long were placed in these different nutrient solutions. After the cultures had grown five weeks it was found that the set grown in solutions that lacked nitrogen had developed a root system similar and equal in length to those obtained from cultures grown in tap water. The tops of the plants grown in the relatively nitrogen-free solutions gained only a few centimeters in shoot length, but the root mass had attained a length of 60–70 cm. for the different cultures of the set. From these results it was concluded that stimulation of long root development of wheat seedlings grown in tap water was related to the deficiency of nitrogen in that growth medium.

FIG. 1.—Culture to left grown in tap water for six weeks; culture to right grown in good nutrient solution for two weeks.

Two questions might be asked in reference to the results obtained: (1) Can plant roots grow without nitrogen? (2) What constitutes the best root development of a wheat plant for its normal growth? As to the first question, the tests did not prove that the large root development obtained from wheat seedlings grown in tap water or in the prepared nitrogen-free solutions was due to the total absence of nitrogen, or that it would have been obtained in the total absence of nitrogen. Obviously some nitrogen was contained in the seedlings when they were set in these media. Presumably less and less became available to the growing roots as the plants grew older, however, as the small supply originally in the seed had to suffice for more and more tissue (chiefly roots) as the seedlings enlarged. Whether the supply was ever exhausted in the growing region of the roots is not known.

An answer to the second question must also be given as a hypothesis. The large root development of the wheat seedlings placed in tap water did not result in the production of large wheat plants. The roots grew at the expense of the tops. Obviously wheat shoots could not have grown to any appreciable extent without roots, so between the two limits thus indicated (no roots on the one hand, and one-half of the total dry matter being roots on the other hand) must be found that relation of root to top that will bring about the best growth of the wheat plant.

Fig. 1 shows the relative root development of two different cultures, with approximately similar top growth. One was grown in tap water for six weeks and produced roots over 100 cm. long, the root mass being about six times longer than the length of the tops. The other culture was grown in a good nutrient solution for two weeks and produced roots that were only a trifle longer than the length of the tops. Approximately this same ratio of length of root to that of top would have been maintained if it had been grown six weeks or longer in this good nutrient solution.—W. F. GERICKE, *Division of Soil Chemistry and Bacteriology, University of California.*

CURRENT LITERATURE

BOOK REVIEWS

Ecological plant geography

The name of WARMING always comes first to mind when one thinks of the great names in modern ecology. In November 1921 he passed his eightieth milestone, receiving a portrait album together with the congratulations of his coworkers in all lands. Ten years ago he retired from active service at the University of Copenhagen, but these ten years have been full of important researches, and his publications during this period have been numerous. One of the most important of these publications is the third German edition of his ecological plant geography.[1] The first German edition appeared in 1896, being essentially an unmodified translation by KNOBLAUCH of the original Danish edition of 1895. The second German edition was issued by GRAEBNER without cooperation with WARMING. The English edition of 1909 was essentially a new book, with a very different grouping of the subject matter, in which the author was materially assisted by a young Danish geographer, MARTIN VAHL. The third German edition has been worked over very carefully by WARMING, although it follows the general features of the English edition. The most conspicuous changes are seen in the chapter that deals with formations and associations, and here the author follows the recommendations of the Brussels Congress of 1910. The book is also much larger than preceding editions, and the references to the literature are brought to date, so far as possible. But for the war, the book would have appeared much sooner than it did. It was asked for by the publishers in 1912, and was ready in 1914.—H. C. COWLES.

Principes de Biologie Vegetale

Following his volume on *L'Evolution des Plantes*, published in 1918, the second posthumous volume of BERNARD'S[2] lecture notes has been published under the title *Principes de Biologie vegetale*. The first part deals with cellular physiology of plants, with chapters on the principle of determinism, physical conditions of nutrition, nutritive metabolism, carbon nutrition, nitrogen nutrition, and the action of exterior agents upon the living cell. The second part of

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[1] WARMING, EUG., and GRAEBNER, P., EUG. WARMING'S Lehrbuch der ökologischen Pflanzengeographie; dritte umgearbeitete Auflage. pp. 762. Berlin: Gebrüder Borntraeger. 1918.

[2] BERNARD, NOEL, Principes de Biologie vegetale. pp. xii+212. *figs. 18*. Paris: Felix Alcan Library. 1921.

the volume is entitled coordination, and contains chapters on Thallophytes and Schizophytes, Myxomycetes and fungi, algae, lichens, and a final chapter on immunity among plants. It is an elementary treatise, written in entertaining and lucid style. That the author has been dead ten years accounts for the appearance of occasional remarks which do not quite reflect our latest knowledge, as for instance, that "the formula for the constitution of chlorophyll is not known." Beginners, either in botany or French, would find it a delightful little volume.—C. A. SHULL.

MINOR NOTICES

Flora of Natal.—BEWS,[3] well known for his ecological study of the vegetation of Natal, has published a taxonomic account of the flora "for the purpose of assisting the study of plant ecology and botanical survey work in Natal." The introduction contains a very interesting account of the history of botany in Natal, from the earliest collector (1832) to the present time. The analytical keys are remarkably simple, leading to the genera, but the species are merely listed, with their ecological range and often with their local Zulu names. The author states that "the flowering plants of Natal, as now arranged, belong to 148 families, and include 901 genera and 3786 species."—J. M. C.

Osmotic pressure.—The publication of a new edition of Pfeffer's[4] famous work on osmotic pressure will be welcomed by students of plant physiology and physical chemistry who have desired to own a copy of this classic work. No changes have been made from the first edition, except that an introductory appreciation of PFEFFER'S work by CZAPEK precedes the text.—C. A. SHULL.

NOTES FOR STUDENTS

Specificity of chromosomes and sex-determination.—For a final proof of the rôle of the individual chromosome we must look to the remarkable investigations of BRIDGES.[5] It was this author who furnished a direct demonstration of the chromosome theory of heredity, when he showed that irregular distributions of the sex chromosomes of *Drosophila* were accompanied by irregularities in the inheritance of known sex-linked factors. He now[6] provides a similar demonstration of the specificity of the autosomes, and at the

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[3] BEWS, J. W., The flora of Natal and Zululand. pp. vi+248. Pietermaritzburg. 1921. 15*s*. (Whelden and Wesley, 28 Essex St., Strand, London).

[4] PFEFFER, W., Osmotische Untersuchungen. pp. xiv+236. *figs*. 5. Leipzig: Engelmann. 1921.

[5] BRIDGES, C. B., Non-disjunction as proof of the chromosome theory of heredity. Genetics 1:1–52. 1916.

[6] ———, Triploid intersexes in *Drosophila melanogaster*. Science 54:252–254. 1921.

same time adds a very significant and far-reaching modification of present ideas on sex determination.

An unexpected distribution in inheritance of known factors which are located on the second and third chromosomes of *Drosophila* was explainable on the assumption that the female parent of the cross was a triploid with respect to these chromosomes. Cytological examination proved that this was actually the case. This same group of flies also exhibited some remarkable irregularities in their sex condition. A considerable group of "intersexes" occurred, as evidenced by the secondary sex characters and the condition of the gonads as well. This was apparently a bimodal group, some of the intersexes being of a more "female type" and others of a more "male type." Cytological examination of these individuals revealed that the second and third chromosomes were regularly present in a triploid condition, that the fourth chromosome was either diploid or triploid, and that two *x*-chromosomes were regularly present (with or without a *y*-chromosome). The situation is interpreted as follows. "It is not the simple possession of two *x*-chromosomes that makes a female, or of one that makes a male. A preponderance of genes that are in the autosomes tends toward the production of male characters; and the net effect of genes in the *x* is a tendency to the production of female characters. The ratio of *2x:2 sets autosomes* produces a female, while *1x:2 sets autosomes* produces a male. An intermediate ratio, *2x:3 sets autosomes*, produces an intermediate condition, the intersex. The fourth chromosome seems to have a disproportionately large share of the total male-producing genes; for there are indications that the triplo-fourth intersexes are preponderantly of the 'male type', while the diplo-fourth intersexes are mainly 'female type.'" According to this conception, *3x:2 sets autosomes* should be "superfemales," and *1x:3 sets autosomes* "supermales." The author has actually identified such types, both being sterile.

It is certain that this conception will exert a far-reaching influence upon the existing ideas of sex-determination. In the first place, it gives a somewhat more exact idea as to the elements effective in determining sex. Hitherto it has been thought, rather vaguely, that the *x*-chromosome determines sex either per se or by virtue of some special factor which it contains. It is interesting to realize that a number of factors may be influencing sex in one direction or the other, and perhaps that these are identical with factors which have previously been known as playing another rôle. A different rate of metabolism has commonly been associated with the two sexes; a study of the influence of specific factors on metabolic rate now becomes significant in this connection. In the second place, it furnishes an exact interpretation of intersexes on a chromosome basis. Hitherto intersexes have either been interpreted in very vague terms, or have been used as an argument against the chromosome theory of sex determination, or have been harmonized with the sex chromosome theory only by the assumption of some additional extrachromosomal influence (GOLDSCHMIDT). The present conception paints a quantitative picture of sex

without calling upon any other effective elements than the "orthodox" factors of inheritance that are located on the chromosomes. In the third place, the theoretical possibility of artificially controlling sex is illuminated. Such control should be possible to the degree that the ordinary heritable characters can be successfully duplicated artificially. The fact that the fourth chromosome (which is known to contain relatively few factors) is preponderant in its influence toward maleness suggests that a few specific factors may be preponderant in influence. Artificial control, therefore, should necessitate the duplication of the effects of only a few of the factors. Also, the identification of particularly effective heritable factors should be followed by the establishment of a race with a heritably distorted sex ratio.—M. C. COULTER.

Taxonomic notes.—BØRGESEN,[7] in continuation of his studies of the marine algae of the Danish West Indies, has completed the Rhodophyceae. These two concluding parts include 101 species, four of which are new, distributed among 29 genera. The following three new genera are established: *Cottoniella*, *Coelothrix*, and *Hypneocolax*. An extensive appendix (86 pp.) gives a list of the Chlorophyceae, Phaeophyceae, and Rhodophyceae found at the islands, together with addenda and corrections.

ENGLER[8] and his collaborators, in continuation of their studies of the African flora, have published the following results: ULBRICH describes 4 new species of *Pavonia;* MEZ describes 94 new species of grasses, 33 in *Panicum*, 33 in *Melinis*, and 18 in *Digitaria;* ENGLER describes 16 new species of Gesneraceae, 14 of which are in *Streptocarpus*, and also establishes a new genus (*Ctenocladus*) of Moraceae; WOLFF describes 19 new species of Umbelliferae and establishes *Caucaliopsis* as a new genus; KRAUSE describes 8 new species of Liliaceae; IRMSCHER describes 7 new species of Begoniaceae; and BITTER, in continuation of his monograph of African *Solanum*, has reached 56 species.

RYDBERG[9], in continuation of his work on the Rosaceae, has presented the roses of the Columbia region, which includes Oregon and Washington, together with British Columbia and northern Idaho. In this region he recognizes 37 species of *Rosa* and nine hybrids.

SCHLECHTER,[10] in reorganizing the classification of *Spiranthes*, recognizes 35 species of *Spiranthes* and establishes 16 new genera as follows, chiefly from Mexico, the West Indies, and South America: *Galeottiella*, *Hapalorchis*,

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[7] BØRGESEN, F., The marine algae of the Danish West Indies. Rhodophyceae (5 and 6). Dansk Botanisk Arkiv **3**: 305–498. *figs. 308–435.* 1919 and 1920.

[8] ENGLER, A., Beiträge zur Flora von Afrika. XLVIII. Bot. Jahrb. **75**: 161–301. 1921.

[9] RYDBERG, PER AXEL, Notes on Rosaceae. XIII. Bull. Torr Bot. Club **48**: 159–172. 1921.

[10] SCHLECHTER, R., Versuch einer systematischen Neuordnung der Spiranthinae. Beih. Bot. Centralbl. **37**: 317–454. 1920.

Beloglottis, Mesadenus, Pseudogoodyera, Brachystele, Schiedeella, Trachelosiphon, Deiregyne, Gamosepalum, Funkiella, Cladobium, Coccineorchis, Lyroglossa, Pteroglossa, and *Centrogenium.*

STAPF[11] has established a new genus (*Daturicarpa*) of Apocynaceae from the Belgian Congo. It belongs to the Tabernaemontaneae, and includes three species of shrubs.—J. M. C.

Classification of symbiotic phenomena.—MCDOUGALL[12] has written a very sensible and stimulating article on symbiosis and its subdivisions. Very properly he disapproves of the numerous restricted definitions of the term, going back to the original definition of DEBARY, which happens also to be the only definition that justifies the retention of the word in the literature, and the only definition that is etymologically correct. It is one of the curiosities of biological science that so many writers have used the term symbiosis in the sense of mutualism, a relationship that does not exist; and even if it did exist we should not need two terms for the same relationship. The term is much needed, however, in the original and correct sense of "the living together of dissimilar organisms," as pointed out by MCDOUGALL, for there is no other term of such broad and general nature. The author's primary division of symbiosis is into disjunctive and conjunctive, each in turn being subdivided into social and nutritive; each type of nutritive symbiosis may be further subdivided into antagonistic and reciprocal. Plant communities illustrate social disjunctive symbiosis; lianas and epiphytes illustrate social conjunctive symbiosis. Antagonistic disjunctive symbiosis is illustrated by herbivores and plants; antagonistic conjunctive symbiosis is illustrated by the ordinary cases of parasitism, such as plant diseases, ectotrophic mycorhizas, etc. Reciprocal disjunctive symbiosis is illustrated by flowers and pollinating insects, reciprocal conjunctive symbiosis by cases of reciprocal parasitism, such as are seen in lichens, root tubercles, and endotrophic mycorhizas. MCDOUGALL condemns the curious view of some botanists that lichens are simply fungi. He asserts that it is just as absurd to call a fungus-alga combination a fungus as it would be to apply the term fungus to the mycorhizal combination of roots and fungi.—H. C. COWLES.

Forests of British Columbia,—WHITFORD and CRAIG have published an admirable volume on the forests of British Columbia, which are among the most interesting forests in existence.[13] The report is based on three years of

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[11] STAPF, O., *Daturicarpa*, a new genus of Apocynaceae. Kew Bull. no. 4. 166–171. *figs. 2.* 1921.

[12] MCDOUGALL, W. B., The classification of symbiotic phenomena. Plant World **21**:250–256. 1918.

[13] WHITFORD, H. N., and CRAIG., R. D., Forests of British Columbia. Rept. Comm. Conserv. Canada, Committee on Forests. pp. 409. *pls. 28. maps 21.* Ottawa. 1918.

careful study, and it forms one of the most satisfactory volumes dealing with forest resources that has come to our attention. The forests of British Columbia are much more important economically than those of any other province; indeed it is thought that the lumber resources of British Columbia are equal to the combined lumber resources of the other provinces. The province has an area of about 356,000 square miles, of which more than half (200,000 square miles) is unsuited to the production of merchantable timber, chiefly because of altitude. Of the 156,000 square miles that might produce timber, 100,000 have been ruined by fire. As a matter of fact the land now clothed with merchantable timber amounts to only 28,000 square miles. Since most of the forest land is non-agricultural, a strong plea is put forth for reforestation. The chapters in Part I deal respectively with geographical relations, physiographic relations, climatic and soil relations, land tenure, forest administration, forest policy, forest exploitation, forest trees, and insect injuries. The physiographic chapter brings out the fact that British Columbia is "a sea of mountains," and that the average altitude of the province is 3500 ft. above the sea. To the ecologist the most interesting chapters are the one on climatic and soil relations, in which are discussed the various forest types of the province, and the one on forest trees, giving a detailed account of each of the tree species. About half of the maps portray the distribution of individual species. The plates exhibit excellent photographic reproductions of forest types and scenes.—H. C. COWLES.

Montane flora of Burma.—In sketching the vegetation of the mountains of northeastern Burma, WARD[14] shows that a tropical rain forest of Indo-Malay forms, such as *Dipterocarpus*, *Shorea*, *Garcinia*, *Calamus*, and *Ficus*, is found up to an altitude of 5000 ft. From 5000 to 8000 ft. there is developed a temperate rain forest, with *Gordonia*, *Quercus*, *Magnolia*, *Acer*, and *Rhododendron* as characteristic species. Epiphytic mosses, ferns, and orchids abound, but lianas are few. There follows a conifer forest extending from 8000 to 12,000 ft., which shows its tropical relationship only by the presence of species of bamboo. *Abies* predominates, with some admixture of *Pseudotsuga*, *Pinus*, *Juniperus*, and *Larix*. *Rhododendron*, with over 50 species in the undergrowth and in the higher alpine scrub, *Ribes*, *Rubus*, *Rosa*, *Philadelphus*, *Deutzia*, and *Hydrangea* are among the most abundant shrubs.

An examination of the flora reveals an admixture of Himalayan, Indo-Malayan, Chinese, and endemic forms. This leads to the conclusion that this mountain barrier, marking the eastern limit of the Indo-Malayan region for 750 miles, has been connected in the north with the Himalayan ranges on the one hand, and with the great China divide on the other, linking them in a common center.—GEO. D. FULLER.

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[14] WARD, F. K., The distribution of floras in southeast Asia as affected by the Burma-Yunnan ranges. Jour. Ind. Bot. **2**:21–26. *pls. 2*. map. 1921.

GENERAL INDEX

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